



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellant(s): Maria Adele Pacciarini et al. **Examiner:** Ganapathy Krishnan
Serial No.: 09/786,998 **Art Unit:** 1623
Filed: June 14, 2001 **Docket:** 17815
For: USE OF METHOXYMORPHOLINO **Dated:** February 1, 2007
DOXORUBICIN FOR THE
TREATMENT OF A LIVER TUMOR

Confirmation No.: 1122

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APPELLANTS' BRIEF ON APPEAL

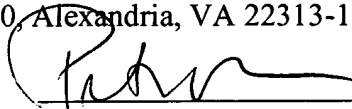
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Dated: February 1, 2007


Peter I. Bernstein

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TABLE OF CONTENTS

(i) Real Party in Interest	2
(ii) Related Appeals and Interferences	3
(iii) Status of Claims	4
(iv) Status of Amendments	5
(v) Summary of Claimed Subject Matter	6
(vi) Grounds of Rejection to be Reviewed on Appeal	9
(vii) Arguments	10
(viii) Claims Appendix	i
(ix) Evidence Appendix	iv
(x) Related Proceedings Appendix	v

(i) Real Party in Interest

The real party in interest of the application on appeal is the assignee,
Nerviano Medical Sciences S.r.l.

(ii) Related Appeals and Interferences

There are no prior or pending appeals, interferences or judicial proceedings known to appellants, appellants' legal representative or assignee which may be related to, directly affect or be directly affected by or having a bearing on the Board's decision in the pending appeal.

(iii) Status of Claims

Claims 1-12 have been cancelled.

Claims 13 and 14 have been finally rejected.

Claims 15-17 have been cancelled.

Claims 18-31 have been finally rejected.

Claims 13, 14 and 18-31 are the subject of the present appeal.

(iv) Status of Amendments

A Request for Reconsideration, mailed November 1, 2006, after final rejection, was not entered.

(v) Summary of Claimed Subject Matter

Independent Claim 13 on appeal is directed to a pharmaceutical composition which includes an active principle methoxymorpholino doxorubicin (MMDX) and a pharmaceutically acceptable agent which remains selectively in a liver tumor after its injection through the hepatic artery. Support for this claim is provided in the specification at Page 9, lines 18-24.

Dependent Claim 14 on appeal is directed to a pharmaceutical composition wherein the pharmaceutically acceptable agent is iodized oil. Support for this claim is provided at Page 9, line 3.

Independent Claim 18 on appeal relates to a method of treating a human liver tumor which comprises the intrahepatic administration of a therapeutically effective amount of MMDX in a patient in need thereof. Support for this claim is provided in the specification at Page 7, lines 16-19.

Independent Claim 19 on appeal is directed to a method of reducing MMDX systemic exposure of a patient suffering from a liver cancer which includes the intrahepatic administration of a therapeutically effective amount of MMDX. Support for this claim is provided at Page 7, lines 11-15.

Dependent Claim 20 on appeal, is directed to a method wherein the liver tumor is primarily confined to the liver. Support for this claim is found at Page 8, line 9.

Dependent Claim 21 on appeal, is directed to a method of treating a human liver tumor primarily confined to the liver which is a hepatocellular carcinoma (HCC) or cholangiocarcinoma. Support for this claim is found at Page 8, line 10.

Dependent Claim 22 on appeal, is directed to a method of treating a human liver tumor metastasis. This claim is supported at Page 8, lines 10-11.

Dependent Claim 23 on appeal, is directed to a method of treating a human liver tumor wherein the intraheptic administration of MMDX is via the hepatic artery. This claim is supported at Page 8, line 13.

Dependent Claim 24 on appeal, whose patentability is separately argued, wherein the MMDX is administered as an infusion of from about 15 minutes to about 30 minutes every 4 weeks to a patient in need thereof, is supported by the specification at Page 7, line 30-Page 8, line 7.

Dependent Claim 25 on appeal, is directed to a method of treating a human liver wherein MMDX is administered as a 5-10 minute bolus every 8 weeks. Support for this claim is found at Page 8, line 17.

Dependent Claim 26 on appeal, is directed to a method of treating a human liver tumor wherein the MMDX is administered with an agent, which remains selectively in a liver tumor after its injection through the hepatic artery. Support for this claim is found at Page 9, lines 1-2.

Dependent Claim 27 on appeal, is directed to a method of treating a human liver tumor wherein MMDX is administered with an agent wherein the agent is iodized oil. Support for this claim is found at Page 9, line 3.

Dependent Claim 28 on appeal, is directed to a method of treating a human liver tumor wherein MMDX is administered in a dose ranging from about 100 mcg/m² to about 1000 mcg/m². Support for this claim is found at Page 8, line 27.

Dependent Claim 29 on appeal, whose patentability is separately argued, is directed to a method of administering MMDX in a dose ranging from about 100 mcg/m² to about 800 mcg/m² is supported in the specification at Page 8, lines 8-10.

Dependent Claim 30 on appeal, is directed to a method of treating a human liver tumor where MMDX is administered at a dose of 200 mcg/m². Support for this claim is found at Page 8, line 29.

Finally, independent Claim 31 on appeal focuses on a method of treating human liver tumor which includes the intrahepatic administration of a therapeutically effective amount of a pharmaceutical composition which comprises as active principle MMDX and a pharmaceutically acceptable agent which remains selectively in a liver tumor after its injection in the hepatic artery. Support for this claim is provided in the specification at Page 7, line 30 – Page 8, line 2 and Page 8, line 2 – Page 9, line 2.

(vi) Grounds of Rejection to be Reviewed on Appeal

(I) Whether Claims 13 and 14 on appeal are patentable, under 35

U.S.C. §103(a), over U.S. Patent No. 5,304,687 to Bargiotti et al. taken with Kuhl et al., Cancer Chemother. Pharma., 33, 10-16 (1993), Nakamura et al., Gan. To Kagaku Ryoho, 8 Pt2, 2562-2567 (August 15, 1988) (English abstract) and Gorbunova et al., Intrahepatic Arterial Infusion Chemotherapy for Primary and Metastatic Cancer of the Liver (1990)?

(II) Whether Claims 18, 20-23, 26 and 27 on appeal are patentable, under

35 U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.?

(III) Whether Claim 19 on appeal is patentable, under 35 U.S.C. §103(a),

over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.?

(IV) Whether Claims 24 and 25 on appeal are patentable, under 35

U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.?

(V) Whether Claims 28, 29 and 30 on appeal are patentable, under 35

U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.?

(VI) Whether Claim 31 on appeal is patentable, under 35 U.S.C. §103(a),

over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.?

(vii) Arguments

(I) Claims 13 and 14 are patentable, under 35 U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.

Claims 13 and 14 on appeal have been finally rejected as being unpatentable over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.

Bargiotti et al. is applied for its disclosure of morpholino derivatives of anthracyclines one of which is methoxymorpholino doxorubicin (MMDX). The morpholino derivatives disclosed in Bargiotti et al. are recited to have utility in inhibiting solid tumors, such as human carcinoma, when applied intravenously or orally. The final rejection admits that the principal Bargiotti et al reference does not disclose intrahepatic administration of MMDX.

Kuhl et al. is applied for its teaching of doxorubicin derivatives. MMDX, one of these doxorubicin derivatives, is taught in Kuhl et al. to have broad-spectrum antitumor activity. MMDX is stated to not being cross-resistant to multidrug tumor resistant models. Kuhl et al. discloses that MMDX is activated in the liver to a greater than 10 times more potent metabolite that cross-links DNA.

Nakamura et al. discloses that the intraarterial infusion of Lipiodol ® (iodized oil) and adriamycin provides positive therapeutic effects for advanced cancer.

Finally, Gorbunova et al. teaches that intrahepatic arterial infusion chemotherapy allows for super high concentrations of an antitumor agent in the organ affected by the tumor.

The final rejection of Claims 13 and 14 on appeal argues that the combined teaching of the above discussed references make obvious to one of ordinary skill in the art a composition comprising MMDX with iodized oil.

Bargiotti et al., the principal reference, indeed discloses morpholino derivatives of anthracyclines of which MMDX is a preferred embodiment. The utility of these compounds is recited in Bargiotti et al. to be antitumor activity in murine animals. Bargiotti et al. discloses that morpholino compounds are introduced into the animals by intravenous or oral administration.

This Bargiotti et al. teaching does not disclose or remotely suggest a pharmaceutical composition which includes MMDX and a pharmaceutically acceptable agent which remains selectively in a liver tumor after its injection. Moreover, this reference does not provide any teaching or suggestion of administering an MMDX composition or indeed any morpholino derivative pharmaceutical composition within the scope of this reference into the hepatic artery.

Kuhl et al. adds nothing to supplement the inadequate teaching of Bargiotti et al. That is, Kuhl et al. provides no teaching directed to the MMDX pharmaceutical composition of Claims 13 and 14 on appeal. Indeed, Kuhl et al., if anything, teaches away from the composition of Claims 13 and 14 on appeal.

Kuhl et al. discloses that MMDX is activated in the liver to produce a metabolite having high potency. However, beyond the disclosure of MMDX having broad spectrum antitumor activity, there is no disclosure of providing a MMDX composition with a pharmaceutically acceptable agent which remains selectively in a liver tumor after its injection through the hepatic artery.

The teaching of Kuhl et al. is away from Claim 13 and 14 on appeal insofar as the Kuhl et al. disclosure of an MMDX composition is limited to the recitation of examples, at Page 11, in which MMDX is disclosed in a composition. That composition, however, combines MMDX with ethanol. That sole composition teaching is away from Claims 13 and 14 on appeal because it is well known to those skilled in the art that such a composition, which is recited to be retained in the liver, will cause cirrhosis of the liver given the notorious biological effect of ethanol in effectuating such an outcome.

The third applied reference, the English language abstract of Nakamura et al., is applied in the final rejection for its teaching of the intraarterial infusion of Lipiodol ® and adriamycin. That combination showed remarkable therapeutic effectiveness against advanced cancer.

Nakamura et al. is directed to a disclosure of clinical trials concerning the use of intrahepatically administered iodized oil and doxorubicin hydrochloride in the treatment of liver tumors. However, Claims 13 and 14 on appeal are directed to MMDX, which is an entirely distinguished compound from doxorubicin (DOX). There is absolutely no mention, teaching or suggestion in Nakamura et al. of the presence or use of MMDX. In view of the selectivity of compounds in the treatment of cancer and the chemically distinguished nature of the compounds DOX and MMDX, the failure to teach or suggest MMDX in Nakamura et al. is evidence of patentable distinction thereover. That Nakamura et al. is substantially concerned only with toxicity and pharmacokinetic data of tumor treatment with Lipiodol ® confirms the non-relevance of this teaching to Claims 13 and 14 on appeal.

It is furthermore emphasized that Nakamura et al. is not combinable with the principal Bargiotti et al. reference insofar as Bargiotti et al. is limited to morpholino derivatives of anthracyclines. DOX, the sole compound within the scope of Nakamura et al., is not a morpholino derivative of an anthracycline.

The last reference applied in the final rejection of Claims 13 and 14 on appeal is Gorbunova et al. Gorbunova et al. discloses only administration and dosage details of DOX in the intrahepatic treatment of liver tumors. Thus, the above remarks directed to Nakamura et al., in emphasizing that references' non-relevance to MMDX, is equally applicable to the Gorbunova et al. reference. Simply put, Gorbunova et al. provides no weight, given its teaching of a chemically distinct compound, in support of the proposition that the MMDX composition of Claims 13 and 14 on appeal are made unpatentable, under 35 U.S.C. §103(a), over its teaching when combined with those of Bargiotti et al., Kuhl et al. and Nakamura et al.

(II) Claims 18, 20-23, 26 and 27 on appeal are patentable, under 35 U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.

Independent Claim 18 on appeal is directed to a method of treating a human liver tumor which includes the intrahepatic administration of a therapeutic effective amount of MMDX to a patient in need thereof.

The above remarks, made in support of the patentability of Claims 13 and 14 on appeal, emphasize that none of the applied references teach the administration of MMDX in the treatment of a human liver tumor wherein a therapeutic amount of that compound is intrahepatically administered to a patient in need thereof.

The primary Bargiotti et al. reference merely discloses MMDX as a promising compound useful in providing antitumor activity in the treatment of murine tumors. No further teaching of that compound in the use defined by Claims 18, 20-23, 26 and 27 on appeal is provided by Bargiotti et al.

The deficiency in the principal Bargiotti et al. reference is evidenced by the application of three secondary references. The first of these, Kuhl et al., merely discloses in vitro activity data suggesting that in tests of human leukemia and lymphoma cell lines MMDX was more sensitive than DOX.

Those skilled in the art are aware that such data as that provided in Kuhl et al. is not definitive of tumor specificity. That is, no disclosure is made in Kuhl et al. evidencing superior tumor reduction in any mammal. Indeed, the only teaching in Kuhl et al. is an in vitro showing of effectiveness against certain blood tumors. One skilled in the art would not thus be presented with a reasonable expectation of success upon using MMDX in the treatment of liver tumors, as required by the claims subject to this ground of rejection. That is, Kuhl et al. only discloses “in vitro” activity of MMDX against blood tumors. This in vitro data only supports the potency of MMDX. The absence of any disclosure of a successful in vivo test evidences the absence of any efficacy in mammals.

It is furthermore emphasized that the previous submission of a reference, “Cancer, Principle and Practice of Oncology,” 6th Ed., DeVita., wherein it is taught that agents useful in the treatment of blood tumors, such as leukemia and lymphoma, have no therapeutic efficacy against solid tumors, further emphasizes the irrelevance of Kuhl et al. to Claims 18, 20-23, 26 and 27 on appeal. Chemotherapeutic agents, such as MMDX, are

tumor-specific and the results of chemotherapy depend on tumor growth characteristics and on the tumor's individual resistance to the drug.

The above remarks establish that the combined teaching of Bargiotti et al. and Kuhl et al. do not make unpatentable, under 35 U.S.C. § 103(a), any of Claims 18, 20-23, 26 and 27 on appeal. That is, the combined teaching of these two references do not so much as suggest treatment of liver tumors by administration of MMDX, let alone intrahepatic introduction of that drug.

When one skilled in the art then turns to the third applied reference, Nakamura et al., the above conclusion regarding the teaching of the first two applied references is unchanged. Nakamura et al. discusses clinical trials concerning the intrahepatic introduction of DOX in the treatment of liver tumors. However, that disclosure is of no consequence insofar as Claims 18, 20-23, 26 and 27 on appeal are exclusively directed to the intrahepatic administration of a therapeutically effective amount of MMDX in the treatment to a patient suffering from a human liver tumor.

In addition to the fact that MMDX is chemically and structurally distinct from DOX and has a distinct and separate status in the art, those skilled in the art also recognize that the two compounds have different biological properties. Indeed, one of the applied references, Kuhl et al., at Page 10, the right hand column under the section "Introduction," states that the value of DOX, although having important therapeutic efficacy in hematologic malignancies, is limited by myelosuppression, cardiotoxicity and development of drug resistance. That these facts prompted the in vitro study of the myelotoxicity of MMDX evidences the totally distinct nature of the two compounds.

That the deficiencies of DOX was the spur to the development of the alternate use of MMDX is evidence of their non-analogous nature.

The last applied reference, Gorbunova et al., provides a teaching substantially the same as Nakamura et al. That is, it teaches the intrahepatic arterial infusion of DOX. As such, the above remarks establishes that no weight, in support of the proposition that Claims 18, 20-23, 26 and 27 on appeal are unpatentable, under 35 U.S.C. §103(a), can be given to Gorbunova et al.

(III) Claim 19 on appeal is patentable, under 35 U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.

Claim 19 on appeal is directed to a method of reducing MMDX systemic exposure of a patient suffering from a liver cancer by the intrahepatic administration of a therapeutically effective amount of MMDX to that patient. It is apparent that this invention is even more remote from the combined teaching of the references applied in the rejection of Claims 18, 20-23, 26 and 27 on appeal than is the invention of those claims.

It is apparent that the remarks, supra, in Argument (I) establish that the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al. does not make obvious the method of treating a human liver tumor by intrahepatic administration of a therapeutically effective amount of MMDX. That is, the applied combined teaching does not make obvious to one skilled in the art the use of the intrahepatic administration of MMDX to a human patient suffering from a liver tumor. In view of this fact, it is apparent that any other effect flowing from that method would not be obvious.

However, even if there were a showing, by the combined teaching of the applied references, suggesting such treatment, the showing of the unexpected result of reduced systemic exposure to MMDX, occasioned by its intrahepatic administration, rebuts any presumption of obviousness flowing from a teaching of MMDX administration.

The experimental protocol provided in the specification of the application on appeal establishes that a reduced concentration of MMDX, an admitted toxic compound, is required to treat liver cancer when administered intrahepatically through the hepatic artery. Thus, even if MMDX treatment of humans suffering from cancer were disclosed in the prior art, which is not the case, still its intrahepatic administration would still be patentable based on the unexpected result of reduced systemic exposure to the toxic chemotherapeutic agent, MMDX.

(IV) Claims 24 and 25 on appeal are patentable, under 35 U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.

Claims 24 and 25 on appeal are directed, respectively, to a method of treating a human liver tumor by the intrahepatic administration of MMDX as an infusion of from about 15 minutes to 30 minutes every 4 weeks or as a 5 to 10 minute bolus every 8 weeks.

The above remarks directed to the patentability of Claims 18, 20-23, 26 and 27 on appeal apply to dependent Claims 24 and 25 on appeal. However, the further treatment regimes required in Claims 24 and 25 on appeal provide yet further grounds for patentability over the final rejection of record.

The showing presented in the specification establish not only the effectiveness of the general method of treatment of human liver cancer but also at concentrations consistent with reduced systemic exposure to MMDX. These inventive concepts are set forth in Claims 18 and 19 on appeal. Claims 24 and 25 on appeal provide specific treatment regimes in accordance with the generalized method of independent Claims 18 and 19 on appeal which provide these unexpectedly improved results. Therefore, these specific methods of treatment, which provide unexpectedly improved results, are patentable, under 35 U.S.C. §103(a), over the combined teaching of the references. This is so even if a prima facie case of obviousness of intrahepatic administration of MMDX in the treatment of human liver tumors were provided. Of course, no such case is presented in the final rejection of record.

(V) Claims 28, 29 and 30 on appeal are patentable, under 35 U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.

Claims 28, 29 and 30 on appeal are directed to the dosage range of the MMDX administered, in the method of treating a human liver tumor, to a patient in need thereof by intrahepatic administration. The difference between Claims 28, 29 and 30 on appeal and Claim 18 on appeal is the inclusion in Claims 28, 29 and 30 on appeal of the specific therapeutic effective amount of the MMDX.

The administration of a dose in the range of from about 100 to about 800 mcg/m² and, more preferably, 200 mcg/m², is, like the discussion above in regard to Claims 24 and 25 on appeal, yet a further distinction evidencing patentability, under 35 U.S.C. §103(a), over the applied references of record. This is so even if the treatment of a

human liver tumor by intrahepatic administration of MMDX were obvious over the applied references of record.

As indicated above, such is not the case. However, even if it were, the dosage range set forth in Claims 28, 29 and 30 on appeal, which is predicated upon both therapeutic effectiveness and minimizing of toxicity problems, would still be a patentable invention in view of the obtaining of these unexpected results.

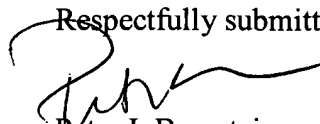
(VI) Claim 31 on appeal is patentable, under 35 U.S.C. §103(a), over the combined teaching of Bargiotti et al., Kuhl et al., Nakamura et al. and Gorbunova et al.

Claim 31 on appeal is directed to a method of treating a human liver tumor which comprises the intrahepatic administration of a therapeutically effective amount of a pharmaceutical composition which includes as active principle MMDX and a pharmaceutically acceptable agent which remains selectively in a liver tumor after its injection through the hepatic artery.

The above discussion emphasizes the non-teaching by any of the applied references of the intrahepatic administration of a therapeutically effective amount of an MMDX pharmaceutical composition. Obviously, this is enough to predicate patentability of Claim 31 on appeal over the combined teaching of these applied references in the final rejection of record. However, it should also be appreciated that the requirement that the MMDX pharmaceutical composition remains selectively in the liver tumor after its injection through the hepatic artery is totally undisclosed in any of the applied references.

This latter requirement, that the MMDX be administered by injection through the hepatic artery, which obviously enhances treatment of the human liver tumor, is also not disclosed or suggested by any of the references cited in the rejection of Claim 31 on appeal.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Peter I. Bernstein', written over the typed name.

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PIB/MB/cl

(viii) Claims Appendix

13. A pharmaceutical composition which comprises as an active principle MMDX and a pharmaceutically acceptable agent which remains selectively in a liver tumor after its injection through the hepatic artery.

14. A pharmaceutical composition according to claim 13. wherein the agent is iodized oil.

18. A method of treating a human liver tumor which comprises the intrahepatic administration of a therapeutically effective amount of methoxymorpholino doxorubicin (MMDX) to a patient in need thereof.

19. A method for reducing methoxymorpholino doxorubicin systemic exposure of a patient suffering from a liver cancer which comprises the intrahepatic administration of a therapeutically effective amount of methoxymorpholino doxorubicin (MMDX) to said patient.

20. A method according to claim 18, wherein the liver tumor is a tumor primarily confined to the liver.

21. A method according to claim 20, wherein the tumor primarily confined to the liver is a hepatocellular carcinoma (HCC) or a cholangiocarcinoma.

22. A method according to claim 18, wherein the tumor is a liver metastasis.
23. A method according to claim 18, wherein the intrahepatic administration of MMDX is via the hepatic artery.
24. A method according to claim 18, wherein MMDX is administered as an infusion of from about 15 minutes to about 30 minutes every 4 weeks.
25. A method according to claim 18, wherein MMDX is administered as a 5-10 minute bolus every 8 weeks.
26. A method according to claim 18, wherein MMDX is administered with an agent, which remains selectively in a liver tumor after its injection through the hepatic artery.
27. A method according to claim 26, wherein the agent is iodized oil.
28. A method according to claim 1, wherein MMDX is administered in a dose ranging from about 100 mcg/m² to about 1000 mcg/m².
29. A method according to claim 28, wherein MMDX is administered in a dose ranging from about 100 mcg/m² to about 800 mcg/m².
30. A method according to claim 29, wherein the dose is 200 mcg/m².

31. A method of treating human liver tumor, which comprises the intrahepatic administration of a therapeutically effective amount of a pharmaceutical composition which comprises as an active principle methoxymorpholino doxorubicin (MMDX) and a pharmaceutically acceptable agent which remains selectively in a liver tumor after its injection through the hepatic artery.

(ix) Evidence Appendix

The following references, enclosed herewith, were introduced into the prosecution file during the pendency of the present application on appeal:

Shepard et al., *Reg. Cancer Treat.*, **3** (4): 197-201 (1990);

Collenoi et al., *Ann. Oncol. (Meaning Abstract)*, **5**(Supp. 8), (1994);

Ono et al., *Semin. Oncol.*, **24**(2 Supp. 6): 6-18-S6-24 (1997);

Lai et al., *Arch. Surge.*, **133**(2): 183-188 (1998);

Chapter 17, Principles of Cancer Management: Chemotherapy V.T.

Devita, J.R. et al.

(x) Related Proceedings Appendix

No related appeals, interferences or judicial proceedings have been rendered which are related to, directly affect or would be directly affected by or have a bearing on the Boards decision. Thus, there are no copies of such decisions enclosed.



Reg Cancer Treat 3(4): 197-201, 1990

Treatment of primary hepatocellular carcinoma by hepatic arterial infusion of 4'-epirubicin (Eng).

Shepherd FA; Rotstein L; Blackstein ME; Burkes R; Erlichman C; Iscoe N; Kutas G; et al:

A group of 23 patients (20 male, 3 female) with hepatocellular carcinoma were treated by hepatic arterial infusion of 4'-epirubicin every 4 weeks. At each treatment, a catheter was inserted percutaneously into the main hepatic artery via the femoral artery under image intensification. Treatment consisted of a 24-h continuous HAI of epirubicin, 30 mg/msup 2/day for 3 days, without heparin. Eleven patients had only one infusion, 4 patients two infusions, 2 patients three infusions, 2 patients four infusions, and 1 patient six and eight infusions each. A partial response was seen in 3 patients, median duration 16 weeks (range 12-46 weeks). Seven patients remained stable, median duration 13 weeks (range 4-38 weeks). The median survival of the overall group was 18 weeks. Survival of responding, stable, and non-responding patients were 38 weeks, 19 weeks, and 10 weeks, respectively. Complications of catheter placement included asymptomatic dissection of the hepatic artery (3 patients), and asymptomatic thrombosis of the hepatic artery (3 patients). Eight patients experienced moderate nausea and vomiting, and 11 patients had moderate to severe alopecia. The granulocyte nadir was above 1000 mul in 83% of evaluable courses, 500-1000 mul in 6%, and less than 500 mul in 11% of courses. Two patients developed neutropenia-associated fever. A platelet nadir below 100,000/mul was seen after only 8% of courses, and only 1 patient had platelets below 50,000/mul. In conclusion, epirubicin has modest activity in hepatocellular carcinoma and is well tolerated when given by hepatic arterial infusion.

(Meeting abstract) (Eng). Ann Oncol 5(Suppl 8)1994

Arterial chemoembolization with epirubicin in unresectable hepatocellular carcinoma in cirrhosis

Colleoni; Gaion; Liessi; Mastropasqua; Nelli; Sgarbossa; Manente:

No reliable therapies have yet been established for unresectable hepatocellular carcinoma (HCC). Systemic chemotherapy with anthracyclines gives less than 20% objective remissions. Encouraging data in terms of response rate and survival have recently been reported with intra-arterial chemotherapy alone or combined with various veno-occlusive materials, specifically ethiolized oil and gelatin sponge; To evaluate the activity and tolerance of a new chemoembolization protocol, patients with unresectable HCC in cirrhosis were treated with epirubicin (50 mg) and ethiolized oil (10-15 ml), administered through hepatic arterial catheters placed percutaneously during angiography, followed by gelatin sponge. Therapy was repeated for a maximum of 3 cycles. Twenty-two eligible patients have entered the study and are evaluable for response and toxicity. Patients were not pretreated with chemotherapy, and only 1 patient had been submitted to surgery. Patient characteristics were: median age 70 yr (range 59-77); ECOG performance status 0-1 in 15 and 2 in 7 cases; Child's A disease in 11 and B in 11; Okuda Stage I in 12 and Stage II in 1 cases; TNM Stage II in 9, Stage III in 3 and Stage IVA in 10 cases. Histologically documented cirrhosis was present in all cases (10 alcohol correlated, 4 Hb-sAg correlated, and 8 HCV related).

A total of 53 courses of therapy have been delivered. Three partial remissions (13%), 2 stabilizations of disease and 17 progressions have been observed. Median time to progression was 4 mo with a median survival of 7.6 mo (range, 1-26+ mo). Significant differences in survival (p less than 0.0001) have been observed between patients at Stage II-III (20 mo) and those at stage IVA (3 mo). The treatment was well tolerated with only 2 cases of WHO Grade I pain and 2 cases of Grade I fever. In conclusion, our results indicate that the schedule has only limited activity in HCC and does not seem to offer any sure advantage over other treatments modalities in HCC.

Semin Oncol. 1997 Apr;24(2 Suppl 6):S6-18-S6-25.

Adjuvant chemotherapy with epirubicin and carmofur after radical resection of hepatocellular carcinoma: a prospective randomized study.

Ono T, Nagasue N, Kohno H, Hayashi T, Uchida M, Yukaya H, Yamanoi A.

Second Department of Surgery, Shimane Medical University, Izumo, Japan.

The intrahepatic recurrence rate is extremely high, even after radical resection of hepatocellular carcinoma (HCC). One report showed intra-arterial administration of epirubicin to be effective in the treatment of nonresectable HCC. We evaluated the effect of postoperative adjuvant chemotherapy including this drug. Fifty-seven patients who had undergone radical resection of HCC were entered in this study. Using the sealed-envelope method, 27 patients were assigned to a group undergoing resection only and 29 patients to a group also receiving adjuvant chemotherapy after resection. The protocol of the chemotherapy was intra-arterial administration of epirubicin (40 mg/m²) at 1 month after resection followed by intravenous administration of epirubicin (40 mg/m²) every 3 months for 2 years. In addition, 1-hexylcarbonyl-5-fluorouracil (HCFU; carmofur), 300 mg/d, was administered orally every day, beginning from 1 month after the resection and continuing (in principle) for 24 months. The clinicopathologic backgrounds were well randomized between the two groups. The chemotherapy protocol was performed completely in only five patients (7.2%). Twenty-four (82.8%) patients had to cease the protocol due to various reasons: change to a new therapy after recurrence of HCC in 19 cases (79.2%), severe side effects of the chemotherapy in three cases (12.5%), liver failure in one case (4.2%), and a postoperative complication in one case (4.2%). The mean total doses were 128 +/- 114 mg for epirubicin and 144 +/- 84 g for HCFU. The cumulative overall and disease-free survival rates for 5 years were not significantly different between the two groups. The results of this prospective randomized study suggest that this adjuvant chemotherapy protocol with epirubicin and HCFU after radical resection of HCC was not effective

Publication Types:

- Clinical Trial
- Randomized Controlled Trial

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Postoperative Adjuvant Chemotherapy After Curative Resection of Hepatocellular Carcinoma

A Randomized Controlled Trial

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Objective: To study the effect of adjuvant chemotherapy after curative hepatic resection in patients with hepatocellular carcinoma.

Design: A randomized controlled trial.

Setting: A tertiary referral center.

Patients: During a 54-month period, 142 patients with hepatocellular carcinoma underwent hepatic resection at 1 institution. Sixty-six patients who survived the operation and had no demonstrable evidence of residual disease on ultrasonographic examination and hepatic angiographic testing at 1 month after surgery agreed to participate in the study. The median follow-up time was 28.3 months.

Intervention: Thirty patients received a combination of intravenous epirubicin hydrochloride (8 doses of 40 mg/m² each at 6-week intervals) and transarterial chemotherapy using an emulsion of iodized oil and cisplatin (3 courses with a maximum dose of 20 mL each at 2-month intervals). Thirty-six patients had no adjuvant treatment.

Main Outcome Measures: Recurrence rate and disease-free survival.

Results: A total of 138 courses of intravenous epirubicin was given to the 30 patients. Sixty-one courses of transarterial chemotherapy were given to only 29 of the 30 patients assigned to the treatment group, because the hepatic artery in 1 patient was thrombosed. Six patients (20%) had chemotherapy-related complications with no mortality. Twenty-three of 30 patients in the treatment group and 17 of 36 patients in the control group had recurrences ($P=.01$). Patients who received adjuvant chemotherapy had a higher incidence of extrahepatic metastases (11 patients vs 5 patients; $P=.03$). The respective disease-free survival rates at 1, 2, and 3 years were 50%, 36%, and 18% for the treatment group and 69%, 53%, and 48% for the control group ($P=.04$).

Conclusion: In a group of patients who underwent curative resection of hepatocellular carcinoma, postoperative adjuvant chemotherapy using the present regimen was associated with more frequent extrahepatic recurrences and a worse outcome.

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ALTHOUGH THE safety of hepatectomy for patients with hepatocellular carcinoma has improved,¹ the prognosis of these patients remains guarded as recurrences are frequent. Depending on the size of the primary tumors, recent reports from Japan,²⁻⁵ France,^{6,7} and Hong Kong¹ showed that recurrent disease could be found in 20% to 64% of these patients within the first year and 57% to 81% at 3 years after surgery. While the hepatic remnant was the predominant site of recurrence, involvement of extrahepatic organs such as lung and bone was frequent.^{4,8} To improve the long-term outcome of these patients after a successful resection, effective measures to reduce the risk for recurrence are mandatory. Preoperative

transarterial chemoembolization has demonstrated no significant benefit and may accelerate deterioration of the already compromised liver function in patients with cirrhosis.⁹ Recent retrospective studies showed encouraging results with the use of postoperative adjuvant chemotherapy in the prevention of recurrent disease.^{2,10-13} Either the transarterial or systemic route was used and various chemotherapeutic agents, including fluorouracil, mitomycin, cisplatin, and doxorubicin and its derivatives had been used in combination or as a single agent. The regimens were extremely varied and questions such as the exact choice and dosage of anticancer agents, optimum timing, duration of treatment, and preferred route of administration remained largely unanswered. We conducted a randomized controlled trial

PATIENTS AND METHODS

Between January 1991 and June 1995, 142 patients with primary hepatocellular carcinoma underwent an elective hepatic resection at our institution. Our technique of hepatic resection has been described previously.¹ At the time of surgery, intraoperative ultrasonography was routinely performed to verify whether all macroscopic disease had been extirpated. For patients with no residual disease in the liver remnant, repeated imaging studies were conducted about 1 month after surgery. These included a percutaneous ultrasonographic examination and a hepatic angiogram. In the absence of any intrahepatic lesions on angiographic examination, iodized oil (Lipiodol, Lipiodol Ultrafluide, Laboratoire Guerbet, Aulnay-sous-Bois, France) was injected into the hepatic artery and this was followed up by a computed tomographic scan of the liver remnant 10 days later. The hepatectomy was considered curative only when these postoperative imaging studies demonstrated no residual tumors.

Seventy-six patients were excluded from this study for the following reasons: previous preoperative chemoembolization (8 patients), gross residual disease at the end of hepatic resection (19 patients), hospital mortality (9 patients), residual disease detected by imaging studies 1 month after undergoing an operation (30 patients), and refusal to participate (10 patients). Sixty-six patients who satisfied the criteria for a curative hepatectomy were enrolled in the study.

There were 53 men and 13 women with a mean age of 53.3 years (range, 28-78 years). The diameter of the tumor was more than 5 cm in 43 patients, 2 to 5 cm in 19 patients, and less than 2 cm in 4 patients. Forty-seven (71%) of 66 patients underwent major hepatectomy. Fifty-six patients (85%) were hepatitis B surface antigen-positive and 36 (55%) had cirrhosis of the liver on histologic examination. The mean interval between hepatectomy and randomization was 50 days (95% confidence interval, 40-59 days). All eligible patients were randomly assigned to receive either no treatment or postoperative adjuvant chemotherapy by drawing sealed consecutively numbered envelopes.

For patients assigned to receive postoperative adjuvant treatment, both systemic and transarterial chemotherapy were started immediately after randomization. Systemic chemotherapy consisted of a maximum of 8 doses of intravenous epirubicin hydrochloride (Pharmacia & Upjohn SPA, Milan, Italy), 40 mg/m² each, administered at 6-week intervals. In addition, 3 courses of transarterial chemotherapy were performed every 2 months via either 1 of the 2 routes. At the end of the operation for 24 patients (12 from each group) undergoing hepatic resection before

September 1993, a cannula connected to a subcutaneous port (Implantofix, B. Braun Melsungen AG, Melsungen, Germany) was inserted into the gastroduodenal artery with its tip at the junction with the hepatic artery. This subcutaneous port provided atraumatic access to hepatic vasculature for angiography or transarterial chemotherapy when necessary. Alternatively, the hepatic artery supplying the liver remnant was selectively catheterized via the femoral artery under fluoroscopic guidance. Using the pumping method, an emulsion consisting of 10 mL of iodized oil and 10 mg of cisplatin (1 mg/mL) was prepared by mixing through a 3-way stopcock from one syringe to another. The emulsion was infused slowly into the hepatic artery until retrograde flow was evident. Intravenous or oral amoxicillin-clavulanic acid and cimetidine were administered immediately before the procedure and for 5 days afterward.

The primary end point was the occurrence of recurrent disease; the secondary end point was survival. The follow-up program was uniform for all patients and included a serum α -fetoprotein assay, chest radiograph, and percutaneous ultrasonographic examination of the liver remnant every 4 weeks for the first year and then at gradually increasing intervals. Suspected recurrent disease was confirmed with appropriate imaging studies and, if possible, histologic or cytologic examination. When recurrence was evident, adjuvant chemotherapy was stopped and the disease treated accordingly with treatment modalities such as reoperation, therapeutic transarterial chemoembolization, or systemic chemotherapy. No patient was lost to follow-up and all follow-up information was updated to May 31, 1996. The study protocol was approved by the Ethics Committee of the Faculty of Medicine of The University of Hong Kong and informed consent was obtained from each patient.

The necessary sample size required was estimated on the assumption that the incidence of recurrent tumor at the end of the third postoperative year for the control and treatment groups was 70% and 35%, respectively. Thirty-one patients were needed in each group to have a type I error of 5% and a type II error of 20% with a 2-tailed test.¹⁴ Comparisons between groups were on an intention-to-treat basis. The statistical tests used included the Student *t* test, the Mann-Whitney *U* test, the χ^2 test with Yates correction, and the Fisher exact test where appropriate. The disease-free survival and survival rates were measured from the day of operation to the time when recurrent tumor was first localized by imaging studies and to the time of death, respectively. Survival was estimated according to the life-table method and was compared using the Wilcoxon test. Statistical significance was $P < .05$; all statistical analyses were conducted using a standard biomedical statistical program (SPSS/PC+, SPSS Inc, Chicago, Ill).

to define the benefits of postoperative adjuvant chemotherapy for patients who had a curative hepatectomy for hepatocellular carcinoma.

RESULTS

Thirty and 36 patients were randomized to the adjuvant chemotherapy and control groups, respectively. The 2 groups were comparable for sex, age, preoperative laboratory data, indocyanine green retention rate, tumor size, extent of resection, and operative blood loss (**Table 1**).

The pathologic features of the resected specimens were also comparable (**Table 2**). The mean interval between hepatectomy and randomization was 47 days for the adjuvant chemotherapy group and 52 days for the control group ($P = .09$).

POSTOPERATIVE TRANSARTERIAL CHEMOTHERAPY

No complications were related to the insertion of the subcutaneous port in all 24 patients (12 from each group).

Table 1. Clinical, Laboratory, and Operative Findings of 66 Patients Studied by Treatment Group*

Finding	Adjuvant Chemotherapy Group (n=30)	Control Group (n=36)
Sex (M/F)	26/4	27/9
Mean age, y (95% CI)†	54.6 (50.2-59)	53.4 (49.2-57.5)
Hepatitis B surface antigen-positive, No. of patients	25	31
Preoperative values		
Median α -fetoprotein titer, ng/mL (range)	246.5 (1-735 000)	181.0 (1-388 800)
Mean serum total bilirubin, μ mol/L [mg/dL] (95% CI)	8.63 [0.5] (7.52-9.75)	13.23 (8-18.45)
Mean serum albumin, g/L (95% CI)	43.7 (42-45.5)	43.8 (42.1-45.6)
Mean prothrombin time, s > control (95% CI)	0.49 (0.29-0.68)	0.63 (0.3-0.96)
Mean indocyanine green retention rate at 15 min, % (95% CI)	11.1 (9.9-12.3)	11 (8.8-13.3)
Tumor size		
Mean, cm (95% CI)	8.5 (6.8-10.1)	10.4 (5.2-15.6)
>5 cm, No. of patients	20	23
Major hepatectomy, No. of patients	23	24
Operative blood loss, L (95% CI)	2.1 (1.6-2.6)	2.3 (1.8-2.8)

*All variables are statistically comparable between the 2 groups.

†CI indicates confidence interval.

The port failed to provide vascular access owing to occlusion or malposition in the early postoperative period in 9 patients (38%), 4 of whom were in the adjuvant chemotherapy group. Three of these 4 patients had successful transarterial chemotherapy performed via the femoral artery, but the remaining 1 had a thrombosed hepatic artery precluding any transarterial injection. Thus, 29 of 30 patients received transarterial chemotherapy via the subcutaneous port (8 patients) or the transfemoral route (21 patients). Fifteen patients received all 3 courses of treatment, while adjuvant transarterial chemotherapy was discontinued in the remaining 14 patients because of recurrent disease (12 patients) and refusal to continue (2 patients).

Three patients had local complications after transarterial chemotherapy via a subcutaneous port. Two patients had cellulitis from extravasation around the port and 1 had severe epigastric pain with necrosis of the lesser curve of the stomach shown on endoscopy. All 3 patients were treated conservatively and, except for 1 who had thrombosis of the hepatic artery, were able to continue treatment via the transfemoral route. There were no other serious adverse effects, such as liver failure, from the transarterial chemotherapy and no patient had any local complications as a result of the femoral artery catheterization.

POSTOPERATIVE SYSTEMIC CHEMOTHERAPY

One hundred and thirty-eight courses of intravenous epirubicin were given to 30 patients in the adjuvant chemotherapy group. Eleven patients received all 8 planned courses but in 19 patients treatment was stopped because of recurrent disease (15 patients), adverse effects (2 patients), and refusal to continue (2 patients). Adverse reactions were recognized in 3 patients during the administration of systemic chemotherapy. One patient with a previous history of thyrotoxicosis had atrial fibrillation and was treated with digoxin. Another patient had leukopenia (lowest white blood cell count, $1.98 \times 10^9/L$) and recovered uneventfully. In both cases, sys-

Table 2. Pathologic Features of 66 Patients Studied by Treatment Group*

Feature	Adjuvant Chemotherapy Group (n=30)	Control Group (n=36)
Cirrhosis	17	19
Multinodular lesion	11	15
Mean macroscopic resection margin, cm (95% CI)†	1.39 (0.96-1.82)	1.45 (1-1.87)
Positive histologic margin	1	5
Venous permeation	14	16
Microsatellite	10	15
Encapsulation	14	24
Capsular invasion	7	14
Stage		
I	1	3
II	9	12
III	20	21

*All variables are statistically comparable between the 2 groups. Stage of tumor is classified according to the description of the Liver Cancer Study Group of Japan.¹⁵

†CI indicates confidence interval.

temic chemotherapy was stopped. The remaining patient had alopecia, which did not affect the schedule of the adjuvant treatment. Thus the overall complication rate for adjuvant transarterial and systemic chemotherapy was 20% (6 of the 30 patients) and there was no treatment-related mortality.

RECURRENT DISEASE AND DISEASE-FREE SURVIVAL

At a median follow-up time of 28.3 months (range, 4.9-77.1 months), 23 of the 30 patients in the adjuvant chemotherapy group and 17 of the 36 patients in the control group had proved recurrent disease ($P=.01$). Recurrence in the liver remnant alone was found in 24 patients, in extrahepatic organs alone in 8 patients, and in both sites in 8 patients (**Table 3**). There was no difference in the incidence of intrahepatic recurrence be-

Table 3. Sites of Recurrence by Treatment Group		
Sites of Recurrence	Adjuvant Chemotherapy Group (n=30)	Control Group (n=36)
Intrahepatic	16	16
Extrahepatic	11	5†
Lung	9	5
Bone	2	0
Total (either site)*	23	17‡

*Four patients from each group had both intrahepatic and extrahepatic recurrences.

† $P=.03$ compared with adjuvant chemotherapy group.

‡ $P=.01$ compared with adjuvant chemotherapy group.

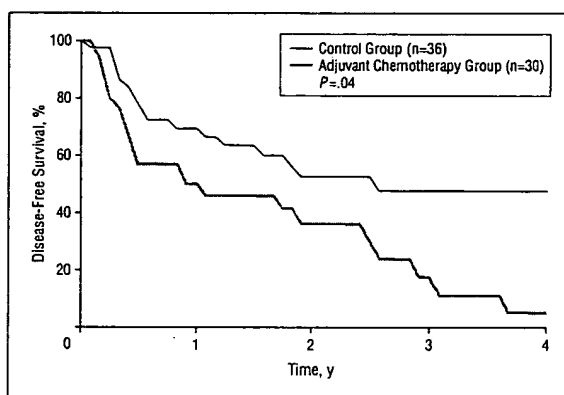


Figure 1. Disease-free survival curves after curative resection of hepatocellular carcinoma. Patients who had adjuvant chemotherapy had a lower disease-free survival than those in the control group ($P=.04$).

tween the 2 groups but patients who received adjuvant chemotherapy had more extrahepatic recurrences (11 patients) than those who were assigned to the control group (5 patients) ($P=.03$). The disease-free survival of the adjuvant chemotherapy group was worse than that of the control group ($P=.04$). The respective 1-, 2-, and 3-year disease-free survival rates were 50%, 36%, and 18% for patients in the adjuvant chemotherapy group and 69%, 53%, and 48% for patients in the control group (Figure 1).

Among the 40 patients with recurrent disease, 12 patients received therapeutic transarterial chemoembolization for intrahepatic recurrence, 22 patients received systemic chemotherapy (with additional external radiotherapy for spinal metastases in 2 patients), and 5 patients were treated symptomatically because of poor performance status. One patient had resection of a solitary pulmonary metastasis followed by intravenous epirubicin treatment and remained disease-free at 3 years after the second operation.

SURVIVAL

At the time of analysis, 10 of the 30 patients in the adjuvant chemotherapy group and 10 of the 36 in the control group had died. The cause of death was progressive recurrent hepatocellular carcinoma in all patients except 1, who died of an unknown cause in the absence of

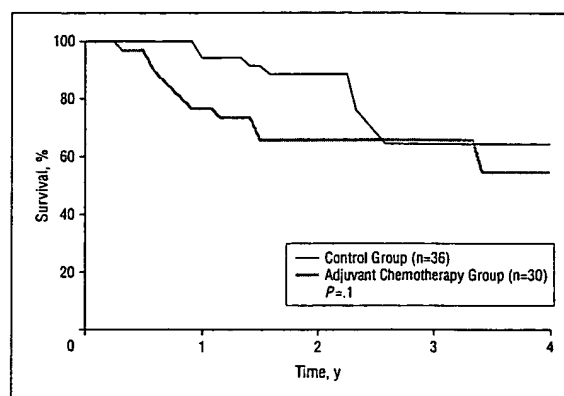


Figure 2. Survival curves after curative resection of hepatocellular carcinoma. The difference in survival between the 2 groups was not statistically significant ($P=.10$).

any evidence of recurrence. The survival of patients assigned to the treatment group was worse than that of the control group (Figure 2), particularly in the first 2 years after the operation, although the difference was not statistically significant ($P=.10$).

COMMENT

When considering postoperative adjuvant chemotherapy that aims primarily at preventing tumor recurrence, the distinction between recurrent disease after a curative operation and residual tumor after a palliative resection is crucial. The curability of hepatectomy for hepatocellular carcinoma is difficult to define. The definition based on tumor staging and resection margin recommended by the Liver Cancer Study Group of Japan¹⁵ is so restrictive that few resections included in the present study could be considered curative. In addition, it does not consider tiny intrahepatic metastases that are not detected by preoperative imaging studies or intraoperative ultrasonography. We have adopted the definition, as described by Nagasue et al,¹¹ that in addition to preoperative and intraoperative findings, a hepatectomy is considered curative only when imaging studies conducted about 1 month after surgery do not reveal any residual disease. Thus, only patients with no demonstrable tumor at the time of randomization are considered suitable for adjuvant chemotherapy, whereas those with residual disease discovered by such screening immediately after operation should be treated therapeutically. Although there are limitations in current liver imaging techniques,¹⁶ a thorough intraoperative ultrasonographic examination followed by repeated investigations using ultrasonography, angiography, and post-Lipiodol computed tomography is regarded as the most sensitive means to confirm the absence of any demonstrable intrahepatic disease before initiation of adjuvant chemotherapy. Even so, more than 50% of the patients in the control group had recurrence at 3 years and the need for adjuvant treatment was justified.

The optimum route of administration, exact regimen, and timing of adjuvant chemotherapy is uncertain. Although the hepatic remnant is the predominant site of recurrence, involvement of extrahepatic organs such

as the lung and bone are frequent.^{4,8} Our previous study of 277 patients who underwent hepatic resections for hepatocellular carcinoma showed that 25.8% had extrahepatic recurrences.⁸ The lower rate of extrahepatic recurrence of 13.9% (5 of the 36 patients) in the control group of the present study can be explained by the definition of a curative hepatectomy, which excludes any patient with residual or recurrent disease within the first month of surgery. Transarterial chemotherapy is an effective locoregional therapy for unresectable¹⁷⁻¹⁹ or recurrent hepatocellular carcinoma²⁰ and recent nonrandomized studies showed that it might reduce intrahepatic recurrences after hepatic resection.^{10,12,13} However, this regional therapy is of no value for extrahepatic tumor dissemination. For adjuvant treatment to be effective, it is conceivable that postoperative chemotherapy should be provided transarterially and systemically.

Based on our experience¹⁷ and that of others¹⁹ in unresectable hepatocellular carcinoma, the response rate of transarterial chemotherapy using an emulsion of iodized oil and cisplatin is between 38% and 55% and may be better than that of treatment using iodized oil and doxorubicin.¹⁹ In the absence of any demonstrable tumor, a maximum dose of 20 mL of the emulsion was considered adequate. Takenaka and associates¹³ recommended postoperative lipiodolization only once or twice, but in view of the high risk for intrahepatic recurrence, the present regimen was intensified to 3 courses of treatment within 6 months. As for systemic chemotherapy, doxorubicin is one of the most active drugs against hepatocellular carcinoma, with a response rate of 10% to 24% in patients with advanced disease.²¹⁻²⁴ Hence, its derivative epirubicin was used because of its reduced cardiac toxic effects. Previous experience with 3 doses of intravenous epirubicin hydrochloride every 3 weeks at full strength (75 mg/m²) after hepatectomy for large tumors showed a high incidence of drug-induced toxic effects, particularly hepatic decompensation.²⁵ We therefore administered epirubicin hydrochloride at half-doses (40 mg/m²) at longer intervals, up to a maximum of 320 mg/m² over 1 year in the present study.

With regard to the timing of adjuvant chemotherapy, Takenaka and associates¹³ started adjuvant transarterial chemotherapy for their patients more than 1 year after surgery. In contrast, other investigators would start at 2 to 6 weeks after surgery and repeat every 3 months for 1 year or longer.^{10,12} The rationale of administering adjuvant chemotherapy after curative resection is to prevent recurrence by suppressing microscopic neoplastic foci. Furthermore, it was reported that recurrence after hepatic resection for hepatocellular carcinoma was most common within the first postoperative year,^{1,7} and this is true even in the present series of selected patients with curative resection. Adjuvant chemotherapy for hepatocellular carcinoma therefore should be started soon after resection. Theoretically, the administration of either regional or systemic chemotherapy soon after hepatic resection may affect the performance status of the patient and depress the regenerative activity of the liver remnant, particularly if there is underlying liver cirrhosis. We withhold chemotherapy for at least the first 4 weeks after the operation and with the present regimen, combined adjuvant transarterial and systemic chemo-

therapy seems to be safe with no serious complications when administered to patients starting 6 to 8 weeks after hepatectomy.

Both surgical cannulation of the hepatic artery followed by placement of a subcutaneous port and transfemoral arterial puncture had been used successfully for delivering chemotherapy intra-arterially.¹⁰⁻¹³ The subcutaneous port was used in the early part of our study because of its theoretical advantage of providing an atraumatic means for repeated access to the hepatic vasculature. Nevertheless, the additional operation time, frequent early occlusion, and morbidity associated with these devices had made the transfemoral route our preferred means for intra-arterial drug administration. The latter route had been employed successfully in all 21 patients without a functioning subcutaneous port with minimal morbidity.

Recent retrospective studies have shown encouraging results with adjuvant transarterial chemotherapy following hepatic resection for hepatocellular carcinoma. Using a combination of fluorouracil, doxorubicin, and mitomycin in Lipiodol delivered transarterially, Nonami and associates¹⁰ found a better survival rate in 19 patients who were treated after the operation than 113 who were not. According to Nagasue et al,¹¹ a significant survival benefit was obtained by giving their patients intravenous epirubicin and peroral fluorouracil after hepatectomy. In a prospective nonrandomized study, Takenaka et al¹³ found a significantly higher disease-free survival in patients who received lipiodolization after hepatectomy than others receiving no treatment, although the timing of their initiation of treatment varied widely from less than 6 months to almost 2 years after the operation. Without a proper control group, these studies had a serious drawback of patient-selection bias. In addition, without proper documentation of a curative resection and the absence of residual disease before initiation of adjuvant treatment, it is difficult to know whether the beneficial effect on survival is merely related to early therapeutic intervention for residual or recurrent disease.

A prospective randomized controlled trial²⁶ showed improved disease-free survival and overall survival with the use of oral 1-hexylcarbomoyl-5-fluorouracil following curative resection for hepatocellular carcinoma. The study, however, involved 26 institutions with a mean of only 2.3 inclusions per institution. The favorable results of this adjuvant chemotherapy trial may be questionable because treatment was suspended owing to adverse effects in 12 (44%) of 27 patients. In contrast, the present randomized controlled study showed that combined transarterial and systemic adjuvant chemotherapy using the present regimen has compromised the disease-free survival and probably the overall survival of a selected group of patients with curative resection of hepatocellular carcinoma. The possibility that angiographic studies performed during transarterial chemotherapy resulted in earlier detection of recurrences and hence a shorter disease-free survival is unlikely. Instead of improving the survival by this early detection of recurrences, the survival of the treatment group was lower, largely because of a higher incidence of extrahepatic metastases and cancer death.

The exact reason for the negative result observed is open to speculation. First, transarterial chemoemboli-

zation was associated with a higher incidence and earlier development of extrahepatic metastases in patients with unresectable hepatocellular carcinoma.^{18,27} The defective blood vessels or the ingrowth of new blood vessels in zones of tumor necrosis may facilitate systemic tumor dissemination. Second, definite evidence shows that malignant primary tumors contain subpopulations of cells that are heterogeneous for metastatic potential and susceptibility to cytotoxic drugs.²⁸ By destroying the subpopulation of drug-sensitive cells, chemotherapy could stimulate the formation of new clonal variants from the surviving subpopulations²⁹ and permit cells with a higher metastatic capability to proliferate. Finally, immune surveillance in control of tumor dissemination may be incriminated. The antimetabolic effect of the present regimen of adjuvant chemotherapy might have depressed the host immunity against tumor metastasis.³⁰

The failure of adjuvant chemotherapy in the present study may call for consideration to intensify the therapeutic regimen. Nevertheless, limitations are inherent in any form of chemotherapy for hepatocellular carcinoma not only because many tumors are slow growing³⁰ and hence cytotoxic drug-resistant, but also because the associated liver cirrhosis limits the maximum tolerated intensity of chemotherapy. Further prospective studies using other regimens are required before the value of postoperative adjuvant chemotherapy can be defined more clearly.

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Surgical Anatomy

The sympathetic trunk is composed of ascending and descending fibers, some of which are preganglionic efferent, postganglionic efferent, and afferent fibers.

CHAPTER 17

Principles of Cancer Management: Chemotherapy

The introduction of chemotherapy in the fifth and sixth decades of the twentieth century has resulted in the development of curative therapeutic interventions for patients with several types of advanced solid tumors and hematologic neoplasms. These advances provided important proof of the principle that anticancer drugs could cure cancer and subsequently resulted in their integration into treatment programs with surgery and radiation therapy in early stages of disease, with excellent results. The important obstacles encountered in the use of chemotherapy have been toxicity to the normal tissues of the body and the development of cellular resistance to these chemotherapeutic agents. During the 1990s, the application of molecular techniques for analysis of the DNA of normal and neoplastic cells began to identify some of the critical mechanisms through which chemotherapy induces cell death. This modern-day technology has also provided insight into the changes within these cells that can confer either sensitivity or resistance to drug treatment. This new level of understanding of the molecular pathways through which chemotherapy works and by which genetic change can result in resistance to drug therapy has opened the door for novel therapeutic strategies in which molecular, genetic, and biologic therapies can be used in combination to attack directly new and specific targets to increase the chemosensitivity of malignant cells to treatment and to protect the normal tissues of the body from therapy-induced side effects. The implementation of such novel therapeutic approaches may provide an important paradigm shift in the manner in which therapy is delivered as we move into the next millennium. Clearly, the long-term goal is to improve the outcome of cancer patients undergoing treatment,

especially in those with neoplasms that currently are resistant to conventional-dose therapy.

HISTORY

The systemic treatment of cancer has its roots in the work of Paul Ehrlich, who coined the word *chemotherapy*. Erlich's use of *in vivo* rodent model systems to develop antibiotics for treatment of infectious diseases led George Clowes, at Roswell Park Memorial Institute in Buffalo, New York, in the early 1900s, to develop inbred rodent lines bearing transplanted tumors that could be used to screen potential anticancer drugs. This *in vivo* system provided the foundation for mass screening of novel compounds.¹ Alkylating agents represent the first class of chemotherapeutic drugs to be used in the clinical setting. Of note, they were a product of the secret gas program of the United States in both world wars. The exposure of military seamen to mustard gas in World War II led to the observation that alkylating agents caused marrow and lymphoid hypoplasia.^{2,3} This observation then led to the direct application of such agents in humans with hematologic neoplasms, including Hodgkin's disease and lymphocytic lymphomas, at the Yale Cancer Center in 1943. However, given the secret nature of the gas warfare program, this work was not published until 1946.^{1,4} The demonstration of dramatic regressions in advanced lymphomas with chemotherapy generated much excitement. At approximately this same time, Sidney Farber reported that folic acid had a significant proliferative effect on leukemic cell growth in children with lymphoblastic leukemia. These observations led to the

development of folic acid analogs as cancer drugs to inhibit folate metabolism; thus, the era of cancer chemotherapy began in earnest.

The cure of childhood leukemias and Hodgkin's disease with combination chemotherapy in the 1960s proved the much-disputed point that a fraction of human cancers, even in their advanced stages, could be cured by drugs. This seminal work laid the foundation for the application of chemotherapy in the treatment of solid tumors. The most disappointing aspect of the work with solid tumors was the failure to cure more patients once it was shown that cancer cells might be more sensitive to cytotoxic drugs than normal cells. This is an especially relevant issue in the adjuvant setting where, because of low tumor burden, cancer cells were thought to be more sensitive to eradication by drug therapy.⁵ In addition, a curious and perplexing distribution of responses to chemotherapy has been noted, in that nearly 90% of all drug cures occur in only 10% of cancer types.⁶

Chemotherapy failure was, at first, thought to be due to variations in tumor growth characteristics. Attention then shifted to the role of specific and permanent mechanisms of resistance to individual chemotherapeutic agents that were either acquired after exposure to cancer drugs or were already present as a consequence of intrinsic genetic mutations within the tumor.⁷ During the 1980s, a new form of multidrug resistance (mdr gene) to a host of natural-product antitumor agents, including the anthracyclines, the taxanes, the vinca alkaloids, and a few antifolate analogs, was identified. This resistance mechanism was believed to play a significant role in the failure of cancer chemotherapy *in vitro*. The mdr gene encodes a 170-kD membrane glycoprotein that acts to extrude these various anticancer agents from the cell, resulting in decreased intracellular drug accumulation. Recently, the mrp gene, a related family member, has been characterized in pleiotropic drug-resistant cancer cells. This gene encodes a 190-kD protein that functions in a fashion similar to that of the p170 glycoprotein to mediate the rapid efflux of drug from the cell.^{8,9} Although mdr and mrp appear to play a key role in the development of drug resistance *in vitro* and *in vivo*, their actual relevance to clinical drug resistance remains unclear.

The therapeutic emphasis in the design of early studies was on maximizing the interaction of the active component of cancer drugs with the cycling cancer cell. The availability of tritiated thymidine, a DNA precursor taken up during the S phase of the cell cycle, made possible the study of the kinetics of cell proliferation. It was quickly determined that cancer cells did not divide faster than normal cells; rather, a larger fraction of the population was dividing.¹⁰ This capacity to enter the cell cycle more frequently was referred to as the *tumor growth fraction*, and it is widely appreciated that cancers, in general, have a significantly higher growth fraction than their normal cell of origin.¹¹ Though the molecular technology at the time was limited, it was clear even then that the essence of a malignant cell was a critical defect in the ability to control its own growth.

Much of the early clinical work in cancer chemotherapy was based on the kinetic modeling of the drug therapy of the murine leukemia L1210 cell line. Cell kinetic studies revealed this system to be an exponentially growing malignancy with a growth fraction approaching 100%.¹² Except for the rare Burkitt's lymphoma, however, a growth fraction this high has no parallel in human solid tumors. For this reason, the mathematics of cell kill based on the L1210 murine leukemic model

could never properly account for the ability to cure some human cancers with a relatively small fraction of cycling cells.³

The work with the L1210 model also was the basis for the long-held dogma that rapidity of growth and frequency of cycling in responsive tumors determined sensitivity to chemotherapy. Thus, slowly growing tumors are not kinetically vulnerable, whereas faster-growing tumors are both responsive and curable. Largely because of their good response to treatment, leukemias and lymphomas were considered to be rapidly growing. This observation led to the odd conclusion that solid tumors, such as lung cancer, colon cancer, and other "resistant tumors," were slow-growing, even though there was insufficient evidence for this. It also ran counter to another important clinical observation: Certain human cancers that display a spectrum of growth patterns from indolent to aggressive become significantly more treatable as well as potentially curable as the cell of origin becomes less differentiated and the growth rate as measured by thymidine-labeling index, increases. When this same tumor transforms, however, to a highly aggressive phenotype, paradoxically it often becomes almost totally incurable.

Non-Hodgkin's lymphoma is an ideal example of this treatment paradox. Diffuse large cell lymphoma (DHL) is a more rapidly growing form of non-Hodgkin's lymphoma that is curable by combination chemotherapy in its advanced stages. Indolent, low-grade lymphomas are more slowly growing tumors than DHL and, while they are highly responsive to treatment they are generally incurable in their advanced form with conventional-dose chemotherapy. Thus, despite a similar cell of origin, the more rapidly proliferating cells are subject to complete eradication by chemotherapy. However, further increases in growth rates within populations of patients with diffuse aggressive lymphomas, as predicted by the degree of expression of the Ki-67 antigen, a nuclear antigen that closely parallels the labeling index, negatively predict for both response to treatment and curability.¹³ This finding suggests that, beyond a certain point, the emergence of drug resistance in some way accompanies an increase in the growth rate of the tumor.

Another important and curious clinical observation not easily explained by the dogma on acquired drug resistance was that normal renewing tissue, such as the bone marrow and gastrointestinal (GI) mucosa, never develop resistance to these drugs. These are the two host tissues that are most commonly affected by most anticancer agents used in the clinic. It is a consistent and disconcerting clinical experience to have a patient's tumor respond to treatment with associated marrow suppression, only to have the tumor grow back in the face of continued treatment while the sensitivity of the marrow to chemotherapy-induced toxicity remains invariant. The same can be said for toxicity to GI mucosa.⁶ It is now well-appreciated that the genetic machinery involved in the cell-cycle checkpoint and apoptosis is preserved in normal host tissues. This fact most likely explains why normal host cells are constantly sensitized to the toxic effect of cytotoxic agents.

CHEMOTHERAPY AS PART OF THE INITIAL TREATMENT OF CANCER

Currently, chemotherapy has a role in four different clinical settings¹⁴: (1) as induction treatment for advanced disease (2) as an adjunct to local methods of treatment, (3) as the pr

mary treatment for some patients who present with localized disease, in whom local forms of therapy by themselves are inadequate, and (4) by direct instillation into sanctuary sites or by site-directed perfusion of specific regions of the body directly affected by the cancer.

The term *induction chemotherapy* has been used to describe drug therapy given as the primary treatment for patients who present with advanced cancer for which no alternative treatment exists.¹⁵ Adjuvant chemotherapy denotes the use of systemic treatment after the primary tumor has been controlled by an alternative modality, such as surgery and radiation therapy. The selection of an adjuvant treatment program for a particular patient usually is based on response rates in separate groups of patients with advanced cancers of the same histologic type. The determination of a population of patients as suitable for adjuvant treatment is based on available data about their average risk of recurrence after local treatment alone. Currently, adjuvant chemotherapy is considered standard treatment for early-stage breast and colorectal cancer.^{16,17} There is also evidence to support the use of chemotherapy after surgical resection of anaplastic astrocytomas.¹⁸

Primary (neoadjuvant) chemotherapy denotes the use of chemotherapy as the initial treatment for patients who present with localized cancer for which there is an alternative but less than completely effective local treatment.^{19,20} For chemotherapy to be used as the initial (primary) treatment of a cancer partially curable by either surgery or radiation therapy, there must be considerable evidence for the effectiveness of the drug program in question against advanced disease of the same type. At this time, neoadjuvant therapy has been effectively used in the treatment of anal cancer, bladder cancer, breast cancer, esophageal cancer, laryngeal cancer, locally advanced non-small cell lung cancer, and osteogenic sarcoma. For some of these tumors, it has now been determined that chemotherapy, when given concurrently with radiation therapy, is superior to sequencing chemotherapy before radiation therapy.

CLINICAL END POINTS IN EVALUATING RESPONSE TO CHEMOTHERAPY

INDUCTION CHEMOTHERAPY

In induction chemotherapy for patients with advanced cancer and measurable disease, it is possible to assess response to drugs on a case-by-case basis. Partial response is usually defined as the fraction of patients who demonstrate at least a 50% reduction in measurable tumor mass. There is growing evidence to suggest that quality-of-life indices are better in patients who show either a response to therapy or a minimal response as compared to supportive care, even if overall survival is not improved. However, partial responses are also useful in the evaluation of new drugs or new drug regimens, to determine whether the particular experimental approach is worthy of further clinical development.

It is clear, however, that the most important indicator of the effectiveness of chemotherapy is the complete response rate. No patient with advanced cancer has ever been cured without first achieving a complete remission. When new programs consistently produce more than an occasional complete remission, they have invariably been proven to be of significant practical

value in medical practice. Thus, in clinical trials, complete and partial responses should always be reported separately. The most important indicator of the quality of a complete remission is the relapse-free survival from the time treatment is discontinued. This criterion is the only clinical counterpart of the quantifiable cytoreductive effect of drugs in the *in vivo* rodent system. The use of freedom from progression in patients who have attained a mixture of complete and partial responses can be misleading when evaluating a new treatment.²⁰ This method of analyzing clinical outcomes is a relatively simple indicator of the practical potential of a new treatment but, for experimental treatments, it obscures the value of a relapse-free survival of complete responders as the major determinant of the quality of remission and the potential for cure. Other end points, such as median response duration and median survival, are also of little practical value until treatment results have been refined so that the complete response rate is higher than 50%.

ADJUVANT CHEMOTHERAPY

There was initially great excitement with the concept of using chemotherapy as an adjunct to local treatment. The rationale for adjuvant chemotherapy was to treat micrometastatic disease at a time when tumor bulk would be at a minimum, thereby enhancing the potential efficacy of drug treatment. It was assumed that drug therapy, at this stage, would result in a much higher cure rate.^{21,22}

The major indicator of effectiveness of a chemotherapy program—the complete remission rate—is lost in the adjuvant setting because the primary tumor has already been removed. In the clinic, treatment is selected for individual patients based on response rates in an entirely different population of patients with advanced disease of the same histologic type. In adjuvant programs, relapse-free survival remains the major end point but, in each patient, micrometastases consist of a mixture of tumor cells, some of which could have been expected to be sensitive to chemotherapy and others of which could have been expected to be resistant to chemotherapy. The relapse-free survival in the adjuvant setting, therefore, measures time to regrowth to clinically detectable levels of cells unresponsive, partially responsive, or very sensitive to chemotherapy and is the equivalent of the duration of remission of a combined group of complete responders, partial responders, and nonresponders. In this sense, it is similar to the use of freedom from progression in patients with advanced disease.

PRIMARY (NEOADJUVANT) CHEMOTHERAPY

The unique feature of using chemotherapy in patients with localized tumor before or in place of purely local treatments, such as surgical excision or radiation therapy (or both), is the preservation of the presenting tumor mass as a biologic marker of responsiveness to the drugs. Moreover, this approach has allowed the sparing of vital normal organs, such as the larynx, the anal sphincter, and the bladder, as the primary tumor is reduced in size and rendered easier to deal with by traditional local measures. As with induction chemotherapy for patients with advanced cancer, it is possible to determine, on an individual basis, the potential efficacy of a new treatment program. A good response to chemotherapy identifies a patient who may benefit from further treatment. A poor response of the pri-

mary tumor to chemotherapy identifies a patient for whom alternative methods of treatment should be seriously considered. Another feature of primary chemotherapy is the ability to differentiate partial responders with varying degrees of prognosis. Removal of residual tumor masses and histologic examination of the tissue allow determination of the viability and character of the remaining tumor cells. The response duration of complete and partial responders must be catalogued separately. Such an approach could result in briefer, less morbid, and more effective treatment programs.¹⁴ When chemotherapy is administered concurrently with radiation therapy, the determinants of sensitivity to drugs are obviated unless compared to radiation alone in a control arm.

The use of chemotherapy as primary treatment is reviewed in each of the appropriate disease-oriented chapters. Table 17-1 lists the specific malignancies in which primary chemotherapy for localized forms of the cancer in question already have been incorporated into clinical usage (first and second categories) and in which current clinical trials show considerable promise (third category).²³⁻³²

PRINCIPLES GOVERNING THE USE OF COMBINATION CHEMOTHERAPY

With rare exceptions (e.g., choriocarcinoma and Burkitt's lymphoma), standard single drugs at clinically tolerable doses have been unable to cure cancer. In the early years of cancer chemotherapy, drug combinations were developed based on known biochemical actions of available anticancer drugs rather than on their clinical efficacy. These regimens were largely ineffective.³³⁻³⁷ The era of effective combination chemotherapy began when an array of active drugs from different classes became available for use in combination in the treatment of leukemias and lymphomas. Combination chemotherapy has now been extended to the treatment of most solid tumors, as described throughout this book.

Combination chemotherapy using conventional cytotoxic agents accomplishes several important objectives not possible with single-agent treatment. First, it provides maximal cell kill within the range of toxicity tolerated by the host for each drug as long as dosing is not compromised. Second, it provides a broader range of interaction between drugs and tumor cells with different genetic abnormalities in a heterogeneous tumor population. Finally, it may prevent or slow the subsequent development of drug resistance.

Certain principles have been useful in the selection of drugs in the most effective drug combinations, and they guide the development of new drug therapeutic programs. First, only drugs known to be partially effective against the same tumor when used alone should be selected for use in combination. If available, drugs that produce some fraction of complete remission are preferred to those that produce only partial responses. Second, when several drugs of a class are available and are equally effective, a drug should be selected on the basis of toxicity that does not overlap with the toxicity of other drugs to be used in the combination. Although such selection leads to a wider range of side effects, it minimizes the risk of a lethal effect caused by multiple insults to the same organ system by different drugs and allows dose intensity to be maximized.

TABLE 17-1. Primary Chemotherapy

NEOPLASMS IN WHICH CHEMOTHERAPY IS THE PRIMARY THERAPEUTIC MODALITY FOR LOCALIZED TUMORS

Large cell lymphoma
Lymphoblastic lymphoma
Burkitt's and non-Burkitt's, undifferentiated lymphoma
Childhood and some adult stages of Hodgkin's disease
Wilms' tumor
Embryonal rhabdomyosarcoma
Small cell lung cancer
Central nervous system lymphomas

NEOPLASMS IN WHICH PRIMARY CHEMOTHERAPY CAN ALLOW LESS MUTILATING SURGERY

Anal carcinoma
Bladder carcinoma
Breast cancer
Esophageal cancer
Laryngeal cancer
Osteogenic sarcoma
Soft tissue sarcoma

NEOPLASMS IN WHICH CLINICAL TRIALS INDICATE AN EXPANDING ROLE FOR PRIMARY CHEMOTHERAPY IN THE FUTURE

Non-small cell lung cancer
Bladder cancer
Breast cancer
Cervical cancer
Esophageal cancer
Gastric cancer
Nasopharyngeal cancer
Other cancers of the head and neck region
Pancreatic cancer
Prostate cancer

Additionally, drugs should be used in their optimal dose and schedule, and drug combinations should be given at consistent intervals. Because long intervals between cycles negatively affect dose intensity (discussed in further detail later in Concept 6 Dose Intensity), the treatment-free interval between cycles should be the shortest possible time necessary for recovery of the most sensitive normal target tissue, which is usually the bone marrow. Finally, there should be a clear understanding of the biochemical, molecular, and pharmacokinetic mechanisms of interaction between the individual drugs in a given combination, to allow for maximal effect.

Omission of a drug from a combination may allow overgrowth by a cell line sensitive to that drug alone and resistant to other drugs in the combination. In addition, arbitrary reduction in the dose of an effective drug to add other less effective drugs may dramatically reduce the dose of the most effective agent below the threshold of effectiveness and destroy the capacity of the combination to cure disease in a given patient.

Most standard treatment programs were designed around the kinetics of recovery of the bone marrow in response to exposure to a cytotoxic agent. The introduction of the colony-stimulating

factors (CSFs) has been a significant advance for cancer therapy, as they help to accelerate bone marrow recovery and prevent the occurrence of severe myelosuppression.³⁸ They play an instrumental role in decreasing the incidence of infections and the need for hospitalizations and allow for maintenance of optimal dose intensity of chemotherapy. Clearly, these cytokine growth factors have revolutionized the next generation of chemotherapy treatment.

Bone marrow has a storage compartment that supplies mature cells to the peripheral blood for 8 to 10 days after the stem cell pool has been damaged by cytotoxic drugs. Events in the peripheral blood are usually a week behind events occurring in the bone marrow. In previously untreated patients not primed by CSFs, leukopenia and thrombocytopenia are observed on the ninth or tenth day after initial dosing. Nadir blood counts are noted between days 14 and 18, with the onset of recovery beginning by day 21 and usually completed by day 28 in patients who have not had prior treatment with drugs or x-irradiation. This sequence may be altered in patients with previous therapy by depletion of the stem cell pool, shortening the time to the appearance of leukopenia and thrombocytopenia and prolonging the recovery time. The interval of greatest importance in the clinic is the duration of the nadir level of leukocytes and platelets. The highest risk of infection or bleeding occurs with granulocyte counts lower than 500/dL and platelet counts lower than 10,000/dL. If this nadir lasts only 4 to 7 days, it is tolerated by most patients without the need for supplemental support. Increasing doses of most anticancer drugs within the range of the maximally tolerated standard dose does not usually ablate the marrow or even prolong the time to recovery; however, it does usually influence the nadir count levels. Repeated dosing during the phase of early recovery of the marrow (days 16 to 21) may result in more severe toxicity in the second treatment cycle in patients whose marrow is not the source of, or involved with, the tumor.

These clinical observations, coupled with the kinetic studies of bone marrow recovery in mice and humans, led to the now-familiar 2-week interval between cycles of the most effective drug combinations, using standard doses without CSFs (new cycles begin on days 21 or 28 after the first dose) to accommodate the recovery time of human bone marrow. Although this treatment schedule is suitable for some tumors, the rapid regrowth of others, such as DHLs, Burkitt's lymphoma, and leukemia, often permit the tumor volume to return to pretreatment levels in the interval required for bone marrow recovery, and other approaches to cycling drug combinations are being explored.

No rigid schedule can accommodate all the variables assumed to be important for maximum effectiveness of combination chemotherapy and the requirements of the patients in the practice of medical oncology. Physicians must often adjust doses at intervals to allow for the safe administration of drugs. The certainty that the therapeutic effect of a drug or drug combination can be lost if the dose or schedule is altered should temper these judgments. Reductions in dose rates also often result in only minimal decreases in toxicity but major reduction in the capacity to attain a complete remission in patients with drug-responsive tumors.⁶ Adherence to the standard sliding scale for dose adjustments, usually published with most new treatments, or the prompt initiation of CSF adjunct therapy is the most appropriate way to optimize the delivery of chemo-

therapy without compromising long-term outcome. The application of appropriate guidelines for dose reductions preserves the intervals between treatment cycles, preserves the integrity of each drug combination and, finally, provides consistency between patients and various clinical studies.³⁹

For many years, clinical trial design has been dominated by the use of alternating cycles of combination chemotherapy. The basis for this study design came from the translation of preclinical experimental data into a model for clinical treatment. In 1943, Luria and Delbruck⁴⁰ observed that the bacterium *Escherichia coli* developed resistance to bacterial viruses (bacteriophage) not by surviving exposure but by expanding clones of bacteria that had spontaneously mutated to a type inherently resistant to phage infection. This was a seminal principle in bacterial genetics that laid the framework for the understanding of the development of spontaneous resistance to cancer chemotherapy.⁴⁰ In 1979, Goldie and Coldman⁴¹ applied this principle to the development of resistance to anticancer drugs by cancer cells without prior exposure to these drugs. They proposed that the nonrandom cytogenetic changes now known to be associated with most human cancers probably were tightly associated with the development of the capacity to resist the action of certain types of anticancer drugs. They developed a mathematical model that predicted that tumor cells mutate to drug resistance at a rate intrinsic to the genetic instability of a particular tumor. Their model predicted that such events would begin to occur at population sizes between 10^3 and 10^6 tumor cells (1000 to 1 million cells), much lower than the mass of cells considered to be clinically detectable (10^9 , or 1 billion cells). The probability that a given tumor will contain resistant clones when a patient's disease is newly diagnosed would be a function of both tumor size and the inherent mutation rate. If the mutation rate is as infrequent as 10^{-6} , a tumor composed of 10^9 cells (a 1-cm mass) would be predicted to have at least one drug-resistant clone; however, the absolute number of resistant cells in a tumor composed of 10^9 cells would be relatively small. Therefore, in the clinic, such tumors should initially respond to treatment with a partial or complete remission but would recur as the resistance clone expands to repopulate the tumor mass. Such a pattern is commonly seen in the clinical setting with the use of chemotherapy in many drug-responsive tumors.

The Goldie-Coldman hypothesis, therefore, predicts that cellular drug resistance should be present even with small tumors and that the maximal chance for cure occurs when all available effective drugs are given simultaneously.⁴¹ Because this would involve using eight to 12 drugs simultaneously, this approach has not generally been tested in the clinic for fear that the use of more than five cytotoxic drugs, at full doses, would not be possible. An alternative approach, using two programs of equally effective, non-cross-resistant drug combinations in alternating cycles, has been under evaluation since the mid-1980s. However, many studies purporting to test the Goldie-Coldman hypothesis have not been properly designed. First, in many instances, inadequate testing has been carried out to determine whether the alternate combination is truly non-cross-resistant and is as effective as the primary treatment. In most instances, these requirements are not met. Second, except in rare instances, dosing is usually not controlled properly. Doses of essential drugs are modified downward, *a priori*, without testing the potential impact of such dose reductions on

outcome. Finally, the requirement for symmetry in biologic characteristics of tumors in different patients is unrealistic. The use of alternating cycles of combination chemotherapy has not yet proven to be more effective than full doses of a single effective combination program.

In 1986, Day⁴² and Norton and Day⁴³ reanalyzed the Goldie-Coldman hypothesis and relaxed the requirement for symmetry in the model. Although it verified the basic tenets of the Goldie-Coldman hypothesis, their model suggested a different approach to sequencing combinations: In many instances, the sequential use of combinations should outperform alternating cycles, because no two combinations are likely to be strictly non-cross-resistant or have equal cell-killing capacity, the symmetry assumed by Goldie and Coldman. Day formulated "the worst-drug rule," which refers to any strategy using more or earlier doses of a treatment that is the least effective of two or more available options.⁶ The worst-drug rule has interesting implications. First, it is a nonintuitive approach. If two treatments—treatments A and B—are available and B is known to be better, a physician is more likely to use B first. Cells that are resistant to the best treatment, B, must be eliminated by the weaker program, A; however, because it is the weaker program, one cannot wait too long to use it or the overgrowth of the population resistant to B will place the physician and patient in a situation that is difficult to overcome. The model predicts that if six cycles of A and B are planned, use of the weaker program, A, first offers a better outcome. There have been clinical examples in which sequential therapies have outperformed alternating cyclic use of the same programs if the dose intensity of the two regimens is carefully controlled.^{44,45}

EFFECT OF THE BIOLOGY OF TUMOR GROWTH ON RESPONSE TO CHEMOTHERAPY

Applying the principles of chemotherapy developed by Skipper et al.^{12,46,47} in leukemia L1210 to the drug treatment of human cancers requires a clear understanding of the differences between the growth characteristics of this rodent leukemia and of human cancers as well as an understanding of the differences in growth rates of normal target tissues between mice and humans. For example, L1210 is a rapidly growing leukemia with a high percentage of cells synthesizing DNA, as measured by the uptake of tritiated thymidine (the labeling index). Because L1210 leukemia has a growth fraction of 100% (i.e., all its cells are actively progressing through the cell cycle), its life cycle is consistent and predictable.⁴⁸

The time to death of animals bearing L1210 leukemia is the interval required to achieve a population size of approximately 10^9 (1 billion) cells. With a growth fraction of 100% and a doubling time of 12 hours, 10^9 cells accumulate by 19 days after the injection of a single cell, by 10 days after the injection of 10^5 cells, and by 5 days after the administration of 10^8 cells. Skipper et al.^{46,47} postulated that the increase in host life span after cytotoxic chemotherapy of L1210 leukemia was largely due to the cytotoxic effect of treatment on the tumor cell population. In these early elegant mouse experiments, they calculated the residual number of cells after treatment by extrapolating back from the duration of prolongation of life after a single treatment. An increase of 2 days in life would be equivalent to a

90% destruction of tumor cells (a 1-log kill), or a reduction the cell number from 10^6 to 10^5 . A 99.999% destruction of tumor cells, a number that seems enormous to most clinicians unless the initial inoculum is small, perhaps 10^4 cells or fewer. If multiple treatments are given, the net tumor cell kill treatment is the sum of the surviving cells plus the regrowth of the tumor cell population before the next treatment.

The cytotoxic effects of cancer drugs in this tumor model follow log cell-kill kinetics. Thus, if a particular dose of an individual drug kills 3 logs of tumor cells and reduces tumor burden from 10^{10} to 10^7 cells, the same dose used at a tumor burden of 10^5 cells reduces the tumor mass to 10^2 . Cell kill, therefore, is proportional, regardless of tumor burden. This model fits the response of L1210 murine leukemia to chemotherapy. When treatment failed in sensitive cell lines, it was because the initial tumor burden was too high to allow delivery of curative doses of chemotherapy to eradicate the leukemia cell. The cardinal rule of chemotherapy—the inverse relation between cell number and curability—established in this model and applies to other model systems. Skipper et al.¹² proceeded to show that this rodent leukemia could be cured by specifically designing doses and schedules of administration that were based on tumor volume and growth characteristics.

Although growth of murine leukemias closely follows exponential cell kinetics, available data suggest that in human solid tumors do not grow in an exponential fashion. For example, the concept of log kill would have predicted that some large tumors in the clinic should have been more sensitive to treatment than has been experienced. *In toto*, experimental data in human solid cancers support a Gompertzian model of tumor growth and regression. The critical distinction between Gompertzian and exponential growth is that in Gompertzian kinetics, the growth fraction of a tumor is not constant but decreases exponentially with time (exponential growth is matched by exponential retardation of growth). The growth fraction peaks when the tumor is approximately 37% of its maximum size. In a Gompertz model, when a patient with advanced cancer is treated, if the tumor mass is larger, its growth fraction is low, and the fraction of cells killed is, therefore, small. An important feature of Gompertzian growth is that response to chemotherapy drug-sensitive tumors depends, in large measure, on where the tumor is in its particular growth curve. Sensitive Gompertzian-growing tumors respond to cytotoxic drugs in a Gompertzian fashion.

Therefore, predictions can be made about the behavior of small tumors, such as the microscopic tumor burdens that might be present after primary surgical therapy. When a tumor is clinically undetectable, its growth fraction would be at its largest and, although the numerical reduction in cell number is small, the fractional cell kill from a known-to-be-effective therapeutic dose of chemotherapy would be higher than later in the tumor course. This observation was initially used to justify dose reductions at lower tumor volumes. However, such unnecessary dose reduction may account for some of the disappointment in the outcome of adjuvant studies in breast cancer. The Gompertzian model for tumor growth is important for another reason: It affects the patterns of regrowth of residual tumor cells. In breast cancer, Norton^{43,49} has analyzed the clinical

cal data from multiple adjuvant studies and from available studies of untreated patients with localized disease.^{6,50} In each clinical study, the Compertzian model precisely fit the growth curves of these tumors. In the adjuvant setting, the model showed that relapse-free survival and survival curves are unable to discriminate between residual cell populations of only one cell and a residual population of 1 million cells, because the regrowth of residual cell populations will be faster at smaller volumes than it will be at larger volumes, producing identical results sometimes at 5 years after diagnosis and treatment. These findings suggest that short of total eradication of micrometastases (cure), varying residual volumes produce similar 5-year relapse-free survival and obscure the major differences in tumor reduction by different programs. This information has been especially useful in the design of new adjuvant treatment protocols for early-stage breast cancer.

APOPTOSIS, CELL-CYCLE CONTROL, AND RESISTANCE TO CHEMOTHERAPY

The kinetic models described are realistic only in the context of a tumor that is sensitive to chemotherapy. Only recently have we arrived at a new understanding of the critical determinants of drug sensitivity and resistance. For more than 30 years, the classic view of anticancer drug action has involved the specific interaction between a given drug and its respective target. Cell death arises as a direct consequence of this drug-receptor interaction. However, the critical molecular mechanisms involved in facilitating the initial coupling of the stimulus to the final response of the cell were never clearly elucidated. With an enhanced understanding of the molecular mechanisms underlying the control of the cell cycle and the process of programmed cell death (apoptosis), it is now clear that this very simplistic model must be reevaluated. In contrast to the drug-target interaction directly leading to cell death as was viewed in the classic model, it is now well appreciated that such an interaction acts as a stimulus to initiate a cascade of events eventually resulting in apoptosis. It is doubtful that cell death induced by chemotherapy occurs by any other mechanism. This pathway involves some type of sensor that detects a death-inducing signal, a signal transduction network, and an execution machinery that facilitates the process of cell death. Moreover, this entire process is exceedingly complex as it is highly dependent on the specific cell type under study, the specific anticancer agent being tested, and the cellular context and environment in which the drug-target interaction is being considered.

In addition, it now appears that the capacity of certain cancers to resist the cytotoxic effects of cancer chemotherapy may be more closely connected to either abnormalities in the genetic machinery of cancer cells or to alterations in the critical pathways of cell-cycle checkpoint control and apoptosis than to the specific mechanisms of resistance unique to each agent. This observation is underscored by the general failure to overcome resistance to chemotherapy in the clinic with approaches that attack only the classic biochemical or molecular mechanisms of resistance (or both). Although this section briefly reviews the complex interrelationship between products of cell-cycle checkpoint genes, oncogenic viruses, transcription factors, apoptosis, and chemotherapy as they relate to drug

resistance, more detailed discussion of these topics are reviewed elsewhere.⁵¹⁻⁵⁹

As noted earlier in the History section of this chapter, normal tissues never develop resistance to chemotherapy. However, one of the most remarkable features of both radiation therapy and chemotherapy, when used to treat sensitive tumors, is that their cytotoxic effects may be initially greater in neoplastic cells than in normal host tissues, including the bone marrow and the GI tract. Doses that eradicate some sensitive tumors will not ablate the bone marrow or destroy the capacity of the GI mucosa to regenerate. No molecular basis for this therapeutic selectivity was available until just recently. However, this difference in cytotoxic action between normal and malignant cells appears to relate to mechanisms that allow normal renewing cell populations, such as bone marrow and GI precursor cells, to monitor and repair damaged DNA or destroy cells with irreparable DNA, rather than allowing damaged cells to proceed through the cell cycle and potentially replicate their damaged DNA. Because they express an intact genetic machinery, normal cells can almost always recover from exposure to DNA-damaging anticancer agents, except in the case of high-dose chemotherapy, as used in transplantation programs. In this particular setting, the high doses of chemotherapy are able to overwhelm these protective mechanisms or to destroy the DNA of exposed cells by direct necrosis (or both). Initially, sensitive cancer cells can be destroyed by effective chemotherapy but, if not, they develop resistance to further treatment, perhaps in part because of drug-induced mutations in their DNA. This resistance may be linked to the dysregulation of the same genetic and signaling pathways that control entry into the cell cycle and the process of programmed cell death.

p53

p53 (Fig. 17-1) is a tumor suppressor protein and critical transcriptional activator that causes both G₁ and G₂ arrest of the cell cycle when cells are exposed to DNA-damaging agents.^{51-54,57,60-62} This function is thought to be critical in preserving the integrity of the cellular genome in response to treatment with a cytotoxic agent. In addition to its role in the cell-cycle checkpoint, p53 is a potent inducer of programmed cell death (apoptosis) within a cell in which DNA damage has occurred.⁵⁰ The basis for the cell's decision either to undergo growth arrest and repair DNA damage or to induce apoptosis remains unknown. Significant research efforts are focused on elucidating the critical factors that determine the eventual cellular function of p53. This is undoubtedly a complex issue, however, that must take into account the extent of DNA damage, the stage of the cell cycle at which the DNA damage occurs, the presence of other genetic abnormalities in either the cell-cycle regulatory apparatus or the signaling machinery, or the specific cellular context. It is now clear that some cell types, such as lymphocytes and the tumors derived from them, have a more rapid access to apoptotic mechanisms than the large majority of epithelial cancers.

Mutations in the p53 gene are among the most common genetic alterations observed in human tumor samples and have been estimated to occur in at least 50% of all human tumors.⁶³ The initial studies showing that loss of p53 function was associated with resistance to radiation therapy as well as chemotherapy came from *in vivo* model systems using p53 knockout mice.⁶⁴⁻⁶⁷ Subsequent studies have confirmed that various

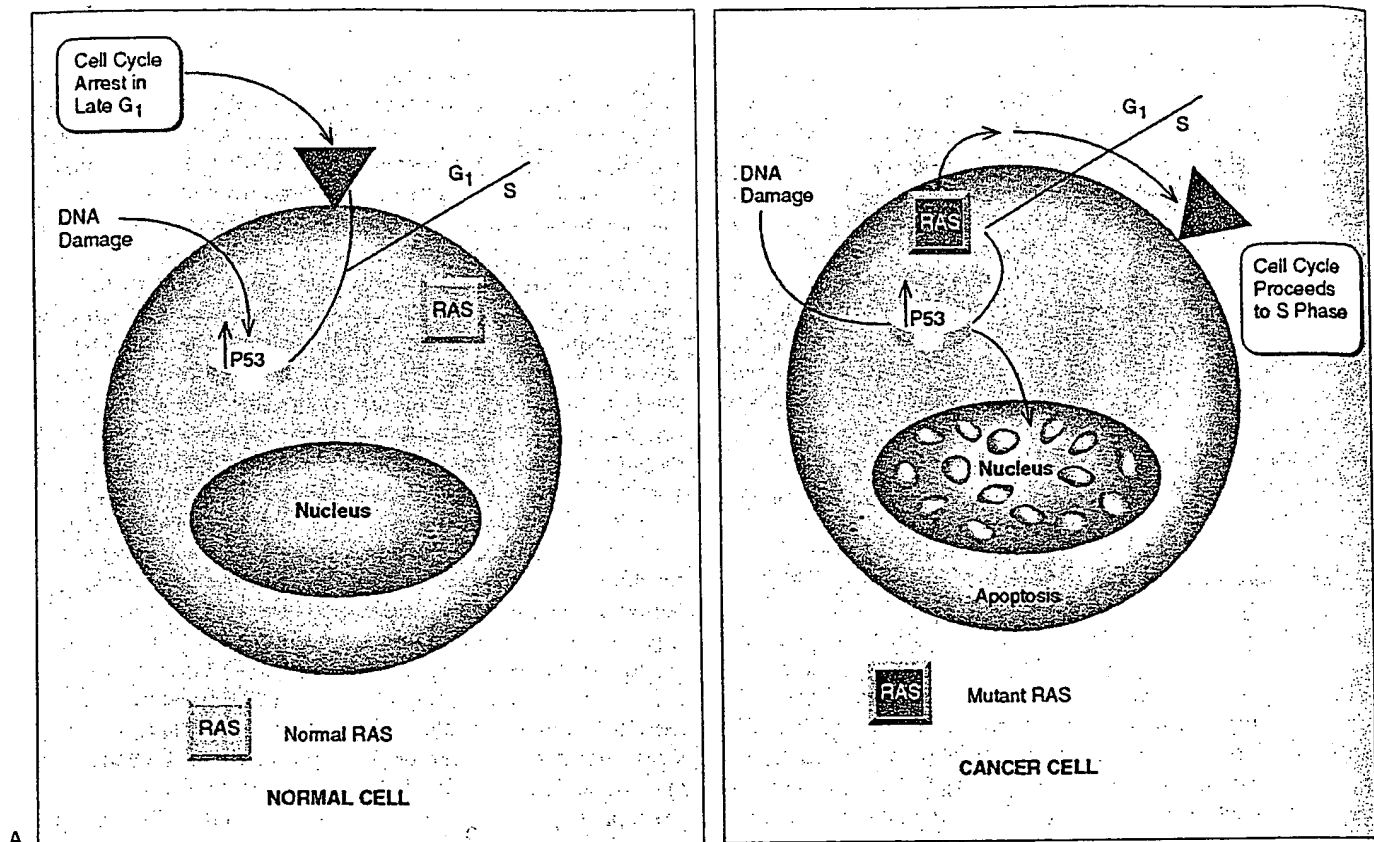


FIGURE 17-1. Role of p53 in chemotherapy sensitivity in normal and neoplastic cells. Exposure of normal cells (A) to DNA-damaging agents results in increased levels of p53, which induces an arrest of progression from the G₁ or quiescent phase to the S or DNA synthetic phase of the cell cycle. Exposure of cancer cells (B) to DNA-damaging agents increases p53 but does not stop cell-cycle progression to S phase, owing to mutant Ras. This results in apoptosis. (From AB Deisseroth, VT DeVita. The cell cycle: probing new molecular determinants of resistance and sensitivity to cytotoxic agents. *Cancer J Sci Am* 1995;1:15, with permission.)

malignant cell lines and tumors expressing mutant or deleted p53 are chemoresistant to a wide range of anticancer agents.⁶⁸ However, loss of p53 function is not always associated with chemoresistance. Some studies suggest that cells with impaired p53 function can become sensitized to various anticancer agents.⁶⁹ Thus, the relationship between p53 status and chemosensitivity is complex and is presumably dependent on a number of factors, including the specific cytotoxic stimuli, tissue-specific differences, and the specific cellular context that incorporates the overall genetic machinery and the various intracellular signaling pathways.

The specific cytotoxic treatment, the conditions of treatment, p53 status, and other cell-cycle regulatory elements may all contribute to the outcome of an exposure of a cell to DNA-damaging agents. If the dose of the treatment is very high, nonapoptotic cell death (e.g., necrotic cell death due to DNA or other damage) may occur. At an intermediate level of dose intensity, p53-dependent or p53-independent apoptotic cell death can occur. When p53 function is intact, the level of inhibitors of p53 is not high, and the regulatory environment of the cell is such that the cell circumvents the interruption of the cell-cycle progression that occurs after DNA damage, the cell will undergo p53-dependent apoptosis. However, in the set-

ting of abnormal p53 function, whether through the acquisition of point mutations in the p53 gene, posttranslation inactivation of p53 through binding to other protein partners (e.g., MDM2) or enhancement of the degradation (e.g., the E6 protein of the human papilloma virus), or decreased translation of wild-type p53 messenger RNA by the folate-dependent enzyme thymidylate synthase, the cell is unable to undergo cell-cycle arrest or apoptosis in response to DNA damage. In a tumor population, the functional inactivation of p53 through any of these regulatory mechanisms facilitates genomic instability and contributes to the development of cellular resistance. Normal hematopoietic cells tend to be more genetically stable during chemotherapy as a result of an intact p53 mechanism that provides an opportunity to repair DNA damage. In contrast, the malignant cell with functional p53 may be more sensitive to chemotherapy than normal cells because of the fact that common transforming mutations in other proteins, such as ras, which tend to drive cells into S phase, overcome the p53-dependent mechanisms that allow for repair. Because the p53-dependent apoptotic mechanisms, triggered by DNA damage, may remain intact, the tumor cell dies after chemotherapy, whereas the normal cell survives. When the function of p53 is finally lost, the stability of the genome of the tumor cells

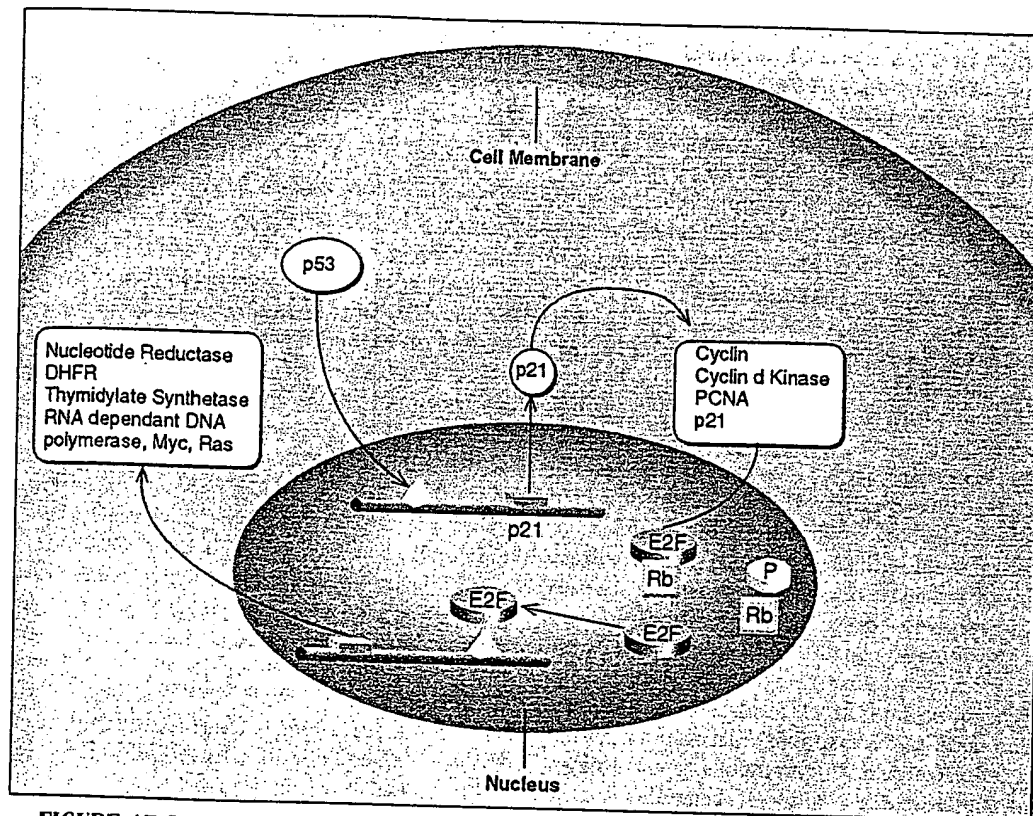


FIGURE 17-2. Once its level is high within the cell, p53 can induce p21 expression to such an extent that the cyclin-dependent kinase activation complex does not occur. The absence of this kinase activity permits the retinoblastoma protein to remain in its unphosphorylated form, in which it binds to the transcription factor E2F. This, in turn, prevents the release of E2F and its binding to the transcriptional enhancers of the genes necessary for DNA synthesis to occur. DHFR, dihydrofolate reductase; PCNA, proliferating cell nuclear antigen. (From AB Deisseroth, VT DeVita. The cell cycle: probing new molecular determinants of resistance and sensitivity to cytotoxic agents. *Cancer J Sci Am* 1995;1:15, with permission.)

decreases, and the disease progresses rapidly to higher and higher levels of resistance to therapy and to a more advanced pattern of dysregulated growth and metastasis.

Although it was initially thought that drug-curable tumors, in general, were less often found to have p53 mutations, this is not always the case. In addition to p53-dependent mechanisms, it is well appreciated that p53-independent mechanisms also exist. In general, the presence of p53 mutations has been correlated with a poor prognosis, even in such treatable tumors as lymphomas.⁷⁰ However, the issue of whether mutations determine cure or no cure will be addressed only by reexamination of the tissue specimens of patients cured many years ago, to separate easily the impact of a damaged cell-cycle checkpoint control system on early response to treatment rather than cure.

This is an important question. If drugs can kill only cells with an intact apoptotic mechanism, as expressed by a functioning p53 gene, the chemotherapy of cancer may have gone as far as it can go in its present form, except for the increment of additional cures that may be attained by using high-dose regimens that overwhelm these mechanisms. If cures are possible in tumors with mutant p53, responsiveness to treatment may relate more to the degree of dysregulation of the checkpoint regulatory pathway, something that possibly can be manipulated as an approach to treatment.

How might dysregulation of this pathway increase drug resistance beyond the failure to induce apoptosis? p53 affects events within the cell by binding to p53 recognition sites located in the transcriptional regulatory regions of genes.⁷¹⁻⁷⁶ The acquisition of point mutations in the p53 gene can affect the DNA-binding function and the transcriptional activation functions of p53 at these regulatory sites.⁵⁹

Some of the genes that are transcriptionally activated by p53 belong to a class of proteins known to inhibit the cyclin-dependent kinases (Fig. 17-2). One of these proteins, known as p21 (Waf-1, Cip-1), can form a complex with proliferating cell nuclear antigen or inhibit the full activation of the cyclin-dependent kinase.⁷⁶ When the cyclin kinase is fully active, it acts on another tumor suppressor, the retinoblastoma (RB) gene, to phosphorylate it.⁷⁷ This causes the release of the E2F family of transcription factors, which then bind to the regulatory regions of a number of genes that participate in the synthesis of DNA. These genes are shown in Figure 17-2 and include ribonucleotide reductase, dihydrofolate reductase, DNA-dependent RNA polymerase, thymidylate synthase, c-myc, c-fos, and c-mb.

Activation of this family of proteins promotes and supports the entry of the cell into S phase. The activation of cyclin-dependent kinases and the consequent turning on of the DNA synthetic machinery by release of E2F from RB occur in normal cells after growth factor stimulation, which probably provides the

signal for the initiation of the cyclin clock.⁷⁸ When normal p53 is activated after DNA damage, the levels of the p21, p27, and other gene products, such as MDM-2, an apparent feedback regulator of p53, and CADD 45, a gene involved in DNA repair, may become very high.⁷⁹ When the expression of p21 is induced to high levels, it exerts an inhibitory effect on the formation of the fully active cyclin kinase complex. This critical checkpoint function of p53, which restricts the procession of the cell into the DNA synthetic phase of the cell cycle, also prevents the E2F-dependent expression of gene products related to rapid cell growth.

The *mdr-1* gene has been added to the list of those potentially influenced by p53 because it has been shown that wild-type p53 suppresses the promoter of the *mdr-1* gene, whereas the mutant protein can actually stimulate the promoter.^{30,31} The biologic basis for this action is not readily apparent but, when the foregoing effects are considered *in toto*, dysregulation of the p53 pathway, which would be expected to be associated with more rapid growth, might well be a prominent mechanism of drug resistance due to the overproduction of gene products responsible for entry into S phase and rapid cell growth. The activation of these genes could theoretically increase the resistance of cells to the following chemotherapeutic agents: methotrexate, 2-chlorodeoxyadenosine, hydroxyurea, fludarabine, cytosine arabinoside, and 5-fluorouracil. Furthermore, the action of an entire array of the most effective natural product antitumor agents could be suppressed through stimulation of the *mdr-1* promoter directly by a mutant form of p53.

Thus, an active p53 in the setting of such DNA-damaging agents as chemotherapy or irradiation increases the levels of key gene products to levels that are sufficient to inhibit the phosphorylation of the RB gene by cyclin-dependent kinase (Fig. 17-3). This, in turn, prevents the expression of the gene products necessary for DNA synthesis to occur.

It is conceivable that increasing growth rates may be associated with increasing levels of drug resistance through the increased transcription of genes involved in rapid cell growth and entry into the cell cycle. The high degree of resistance in more advanced tumors, including the spontaneous development of resistance, which was the basis of the Goldie-Coldman hypothesis, and the development of multi-drug resistance, appear more likely to be related to mutations in key genes in the cell-cycle regulatory system than to drug-specific spontaneous mutations, as has been proposed in the past. Cell death in response to exposure to DNA-damaging agents may require an intact p53-dependent apoptotic mechanism under some experimental circumstances. On the other hand, it also may depend on the activation of alternative pathways of apoptosis or some degree of reregulation of the system which would ultimately lead to the reduced release of transcription factors from genes such as RB, or a homologous gene, p107, and the production of lower levels of growth-related gene products, thereby sensitizing cells to chemotherapeutic agents. An enhanced understanding of the complexities surrounding chemotherapy-induced cell death may shed light on critical insights with profound implications for the design of future approaches to therapy that might couple standard cytotoxic agents to new biologic agents that attack specific molecular targets to reregulate the cell-cycle checkpoint.

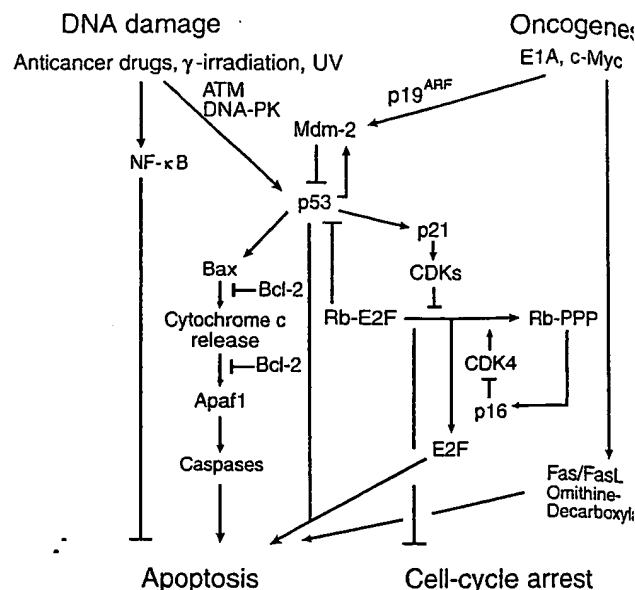


FIGURE 17-3. DNA damage induced by anticancer drugs and irradiation and oncogene expression initiate pathways involved in apoptosis or cell-cycle arrest (or both). CDKs, cyclin-dependent kinases; UV, ultraviolet. (From ref. 56, with permission.)

ROLE OF Bcl-2 FAMILY IN APOPTOSIS

Because apoptosis is a genetically programmed event, inactivation of genes that induce the apoptotic program or activation of antiapoptotic genes can result in the development of cellular drug resistance. Bcl-2 is a potent suppressor of apoptotic cell death, and a number of studies have shown that it is able to repress cell death triggered by either γ-irradiation or a variety of anticancer agents (see Fig. 17-3).^{54,56,82} In further support of the role of Bcl-2 as an inhibitor of cell death are studies that show that treatment of certain human leukemia or lymphoma cells with an antisense strategy directed against *bcl-2* leads to the reversal of chemoresistance. Lymphoma cells treated with either antisense oligonucleotides or with plasmid constructs that overexpress antisense *bcl-2* messenger RNA are sensitive to the cytotoxic effects of methotrexate and cytosine arabinoside.⁸³ In addition, the phosphorylation status of bcl-2 may play an important role as a determinant of chemosensitivity. It has been suggested that phosphorylated bcl-2 may interact less efficiently with its heterodimer protein partner bax, resulting in cell death. Some work suggests that Bcl-x_L, a functional structural homologue of Bcl-2, is also able to confer protection against radiation-induced apoptosis as well as against a number of anticancer agents, including bleomycin, cisplatin, etoposide, and vincristine. Recently, the antiapoptotic effects of Bcl-2 and Bcl-x_L were compared. Using FL5.12 lymphoma cells, it was shown that these two proteins have a differential ability to protect against chemotherapy-induced cell death. This differential effect depends more on the molecular mechanism targeted as opposed to the cell-cycle specificity of an individual drug. In contrast to Bcl-2 and Bcl-x_L, other family members, including Bax, Bcl-x_S, and Bak, have been shown to promote apoptosis in response to either radiation or various anticancer drugs (or both). The underlying mechanism through which these Bcl-2 family members control apoptosis remains unknown at this time. However, the mitochondria

pathway with altered release of cytochrome C presumably plays a critical role in this process.

ROLE OF STRESS-ACTIVATED PROTEIN KINASE IN APOPTOSIS

The mitogen-activated protein kinase family of proteins is highly conserved among all eukaryotic species.⁸⁵ These kinases play a critical role in mediating signal transduction pathways that are sensitive to extracellular stimuli. Stress-activated protein kinase (SAPK), also known as *c-Jun N-terminal protein kinases*, represents one of the key mitogen-activated protein kinase-signaling pathways. It is now well appreciated that SAPK activation is necessary for cell death in response to exposure to certain forms of cell stress and that defects in SAPK signaling promote cell survival. With regard to chemotherapy, SAPK functions are an important mediator of apoptosis.⁸⁶⁻⁸⁸ Inhibition of SAPK activation has a protective effect against cancer cells treated with various anticancer agents, including the anthracyclines and etoposide. In addition, SAPK is required for ceramide-induced apoptosis, a key mediator of cytotoxicity induced by various cancer drugs.⁸⁹ Thus, the SAPK-signaling pathway plays an essential role in facilitating chemotherapy-induced apoptosis. The precise mechanism by which the SAPK pathway is actually activated remains the focus of much research. However, one potential clue rests with the observation that DNA damage induced by genotoxic stress is sensed by kinases, including the DNA-dependent protein kinase or the ataxia-telangiectasia-mutated gene product (or both).^{90,91} These proteins can then phosphorylate p53, resulting in its activation. In addition, DNA-dependent protein kinase can stimulate c-Abl tyrosine kinase, which in turn leads to direct activation of SEK-1, an upstream signal in the SAPK cascade.⁹²

DEATH EXECUTIONER PATHWAY

The molecular mechanisms and signal transduction pathways initiated by a given cellular stress may differ significantly. However, the final stage of these various death pathways occurs through the activation and function of the caspases (see Fig. 17-3).⁵⁹ The caspases represent a conserved family of cysteine proteases with specificity for aspartic acid residues in their substrates. The cleavage of certain essential substrates then results in cell death. Exogenous inhibition of caspase proteases by CrmA promotes the development of resistance of human leukemia cells to a broad array of anticancer agents.⁹³ In addition, some knockout mouse models with germline disruptions of Apaf1, caspase-3, or caspase-9 have shown these genetically engineered mice to be resistant to γ -irradiation and a wide number of chemotherapeutic drugs.^{59,94,95}

CELL SURVIVAL PATHWAYS

It has been shown that a number of external stimuli, including various cytokines, tumor necrosis factor- α (TNF- α), chemotherapy, and radiation, lead to activation of the transcription factor NF- κ B.⁹⁶ Paradoxically, activation of NF- κ B results in potent suppression of the apoptotic potential of these stimuli.⁹⁷ Several studies have demonstrated that inhibition of NF- κ B *in vitro* leads to enhanced apoptosis in response to different stimuli.⁹⁸ Some *in vivo* work shows that the adenoviral delivery of a modi-

fied form of I κ B α , an inhibitor of NF- κ B, results in inhibition of NF- κ B expression. Moreover, the chemoresistant fibrosarcoma tumors derived from HT1080 cells become sensitized to the apoptotic potential of TNF- α and the topoisomerase I compound, CPT-11, leading to significant tumor regression.⁹⁹ These findings suggest that activation of NF- κ B expression in response to chemotherapy may represent an important mechanism of inducible tumor chemoresistance. Moreover, they suggest that strategies to inhibit NF- κ B may lead to enhanced antitumor therapy through increased apoptosis.

DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES

Significant efforts continue to be placed on elucidating the critical intracellular signal transduction pathways required for cell-cycle control and the induction of apoptosis and cell death. However, based on current knowledge, attempts are already being made to manipulate these intracellular programs so as to design and develop novel therapeutic approaches to improve the efficacy of chemotherapy. Tumor suppressor genes were originally identified because of the ability of transforming viruses to bind to the protein product of the tumor suppressor gene and induce the growth required for the virus lytic growth cycle. It has been shown that some viral gene products, such as the adenovirus E1A protein, which by itself is non-transforming, can actually sensitize cells to agents that induce apoptosis. E1A is involved in the release of E2F from the RB protein, which could be expected to facilitate entry of cells into S phase. It has been hypothesized that the transmission of conflicting signals, which act both to slow and to stimulate growth simultaneously, may lead to E1A-induced apoptosis.¹⁰⁰ Thus, the identification of analogs of E1A to be used in this setting is an attractive concept.

Clearly, it will be important to identify novel strategies that are selective in their targeting of malignant cells while preserving normal function of host tissues. Along these lines, viral vectors expressing apoptotic genes can now be directly introduced into the tumor. Adenoviral vectors that express p53 have been injected into human tumors, resulting in suppression of tumor growth.^{101,102} Moreover, this gene therapy approach shows a synergistic interaction with various cytotoxic agents to produce enhanced antitumor activity with minimal host toxicity. This strategy has been taken into the clinic where a phase I study was performed in patients with non-small cell lung cancer.¹⁰³

TNF-related apoptosis-inducing ligand, or TRAIL, is a type II transmembrane protein that was initially identified based on the homology of its extracellular domain with CD95L. Like TNF and Fas, TRAIL induces apoptosis in a wide range of tumor cells.¹⁰⁴ Walczak et al.¹⁰⁵ evaluated the ability of TRAIL as a therapeutic agent. They created leucine zipper (LZ) forms of human (hu) and murine (mu) TRAIL to promote trimerization and found that these trimers of LZ-TRAIL effectively killed cultured mammary adenocarcinoma cells but not normal, non-transformed mammary epithelial cells, fibroblasts, renal tubule cells, skeletal muscle cells, pulmonary epithelial cells, and melanocytes. Administration of either the hu or mu TRAIL was nontoxic to the normal tissues of mice *in vivo*. Finally, repeated administration of LZ-huTRAIL showed impressive antitumor activity against human mammary adenocarcinoma MDA-231 cells implanted in severe combined immunodeficient mice, and

histologic examination of tumors revealed clear areas of apoptosis within 9 to 12 hours of injection. These findings show that TRAIL exerts potent antitumor activity *in vivo* by selectively and directly activating tumor cell death without affecting normal tissues. Studies are ongoing to determine whether TRAIL-based therapy may be used either alone or in combination with other cytotoxic agents and whether these promising results can be effectively translated into the clinic.

It has been suggested that initiation of the cyclic production of cyclins in the cell is due, in part, to the effect of growth factors. Some cyclin classes, such as the D cyclins, may indeed be the essential sensors for multiple growth factor signals.⁶⁰ In some experimental systems, a determining factor in the decision of a cell either to undergo cell-cycle arrest and repair damaged DNA or to undergo apoptosis may be the presence of key growth factors within the cellular environment.^{106,107} Thus, in the absence of a growth signal, with growth factors serving as a survival factor, the cell becomes committed to the apoptotic pathway. It is presumed that apoptosis is taking place within the context of an intact p53 mechanism. However, one issue is whether, even in the presence of mutations of p53 or other checkpoint genes (or both), deprivation of critical growth factor signals would still result in enhanced sensitivity to chemotherapy.

Experiments using *in vivo* mouse models bearing human tumor xenografts suggest that this may be so. An antiepidermal growth factor receptor, monoclonal antibody C225 targeted to the epidermal growth factor receptor, was used in combination with the anticancer agent doxorubicin against human A431 squamous carcinomas and human MDA468 breast carcinomas. The effects of either the antibody or the drug alone are modest, with no long-term survivors. However, when the antibody is administered before doxorubicin, the effects of the combination are dramatic, often leading to complete tumor regression and long-term survival.¹⁰⁸ Similar results were observed in mice bearing human GEO colon cancer xenografts. Complete tumor regression was noted only in mice treated with the topoisomerase I inhibitor topotecan and the C225 monoclonal antibody.¹⁰⁹ These effects at first seem paradoxical, as the cytotoxic effects of an anticancer drug are being enhanced by a biologic agent that presumably is slowing down growth. However, an alternative explanation is that treatment with the antibody sensitizes the tumor to chemotherapy by depriving it of a vital growth factor signal, thereby facilitating the process of apoptosis. The results are provocative enough that other monoclonal antibodies that block key growth factor receptors have been coupled with chemotherapy and are being tested in the clinic. A similar approach is being taken with the use of the anti-HER2 (Herceptin) antibody for the treatment of advanced breast cancer, in combination with either paclitaxel (Taxol) or with the combination of cyclophosphamide (Cytosan) and doxorubicin (Adriamycin).^{110,111} Based on the promising clinical results, these combination regimens have been approved by the U.S. Food and Drug Administration for women with advanced breast cancer.

CONCEPT OF DOSE INTENSITY

Irrespective of the molecular mechanisms underlying the development of human cancers, a principal factor limiting the capacity to cure is proper dosing. The dose-response curve in

biologic systems is usually sigmoidal in shape, with a threshold lag phase, a linear phase, and a plateau phase. For radiotherapy and chemotherapy, therapeutic selectivity is dependent on the difference between the dose-response curves of normal and tumor tissue that must be exploited during treatment. In experimental models, the dose-response curve is usually sigmoidal in the linear phase. Almost without exception in rodents treating transplantable tumors, a reduction of dose in the linear phase of the dose-response curve usually results in a loss of capacity to cure the tumor effectively before a diminution of the response rate is seen. Thus, although complete remission can continue to be observed with a dose reduction as small as 25%, the residual tumor cells may not be eliminated, allowing an eventual relapse to occur. There is an extremely important lesson in these animal data for clinicians who, in their daily practice, judge the adequacy of their therapy by measuring the response rate of visible or palpable tumor masses and much later are able to evaluate the treatment by surgical results. This point is nicely illustrated in Table 17-2, which summarizes data from numerous experiments conducted with Skipper¹¹² at the Southern Research Institute using the transplantable and palpable Ridgway osteosarcoma tumor model. Reduction in the average dose intensity of the two-drug combination of L-phenylalanine mustard and cyclophosphamide causes a marked decrease in the cure rate before a significant reduction in the complete remission rate occurs. On average, a dose reduction of approximately 20% leads to a loss of 50% of the cure rate. The converse is also true. In tumors with a large growth fraction, a twofold increase in dose often leads to a fold increase (1-log) in tumor cell kill. Although *in vivo* systems are not the perfect model for human malignancies, the invaluable nature of these data indicates that the general principle may be applied to the clinic. Because anticancer drugs are toxic, it is often appealing to avoid acute but not life-threatening toxicity by either reducing the dose or increasing the time interval between each cycle of treatment. This kind of empirical dose adjustment is a major reason for treatment failure in patients with drug-sensitive tumors who are undergoing intensive chemotherapy.

One problem facing clinicians is the difficulty in adequately comparing the impact of different dosing practices on the

TABLE 17-2. Ridgway Osteogenic Sarcoma: Response to Different Dose Intensity of Two-Drug Combination of Cyclophosphamide and L-PAM

Relative Dose Intensity			Complete Response Rate (%)	Cure Rate (%)
CPA	L-PAM	Average		
0.38	0.82	0.60	100	60
0.75	0.18	0.47	100	44
0.25	0.55	0.44	100	0
0.50	0.12	0.31	10	0
0.17	0.36	0.27	0	0

CPA, cyclophosphamide; L-PAM, L-phenylalanine mustard.

Note: Tumors weighed 2–3 g.

(Modified from HE Skipper, Booklet no. 5. Birmingham, AL: Southern Research Institute, 1986.)

ical efficacy of chemotherapy. To approach this issue, Hryniuk et al.¹¹³⁻¹¹⁸ analyzed treatment outcomes in various tumor types as a function of dose intensity. Dose intensity is defined as the amount of drug delivered per unit of time, expressed as milligrams per square meter per week, regardless of the schedule or route of administration. Relative dose intensity (RDI) is the amount of drug delivered per unit of time relative to an arbitrarily chosen standard single drug or, for a combination regimen, the decimal fraction of the ratio of the average dose intensity of all drugs of the test regimen compared with the standard regimen. A sample calculation of the RDI for a commonly used regimen, the cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) combination for breast cancer, is provided in Table 17-3.⁷³ To calculate the average RDI for a regimen containing fewer drugs than the standard regimen, a dose intensity of zero is assigned to the missing drug, and the average RDI of the test regimen is divided by the total number of drugs in the standard.¹¹⁴ The dose intensity of each drug regimen is then determined based on the time period in which the treatment program is administered. Specific calculations can be made of the intended dose intensity, the dose intensity as described in the treatment protocol, or the actually received dose intensity. However, determination of the received dose intensity would seem to be the most clinically relevant as it reflects the direct impact of dose reductions and treatment delays imposed in actual practice.¹¹⁸ A positive relation between dose intensity and response rate has, in fact, been demonstrated in several solid tumors, including advanced ovarian, breast, lung, and colon cancers, and in the lymphomas.^{113,114,116-118}

Because dose intensity is determined based on the amount of drug given per week, regardless of schedule, treatment

delays are given equal weight with dose reductions. Calculations of the dose intensity, therefore, assume that differences in scheduling do not influence treatment outcome. Although this concept may at first appear to be contradictory, close scrutiny of all available data in humans and rodents reveals that the schedule of administration influences outcome mainly by affecting toxicity. In this way, higher doses of drug can be administered over the same time frame. As one example, daily administration of low doses of methotrexate is extremely toxic and severely limits the dose and duration of therapy with this drug. However, a twice-weekly schedule, which is much more effective in rodents and humans, allows much higher doses to be delivered over a longer period. Of note, this particular schedule is associated with significantly less host toxicity. As calculated, the dose intensity of the twice-weekly schedule is much greater than that of the daily oral schedule. In practice, the impact of scheduling on the calculation of dose intensity can be neutralized by comparing programs in which drugs with toxicities affected by scheduling, such as the antimetabolites, are given on similar schedules.

One of the potential limitations of the dose-intensity concept is that calculations of an average RDI of a drug combination assume that each drug has equal efficacy against the tumor being treated. In most clinical settings, this is usually not the case. However, the impact of a single drug or combinations of two or three drugs in a multidrug combination can be assessed separately. Such an analysis has been performed to show the greater impact of cisplatin in a drug combination for ovarian cancer¹¹³⁻¹¹⁶ and to show the importance of adequate doses of alkylating agents and vinca alkaloids in lymphoma treatment. The most active drug in a combination regimen can be identified, and this information is important because such data can help to avoid dose adjustments that may radically alter the clinical outcome. Moreover, by identifying the most essential drugs in a given regimen, protocols can focus on administering the optimal dose intensity of those specific agents.

To judge adequately the dosing of a particular protocol, data on total dose of each drug used and cumulative doses of each drug are necessary. However, the collection of such information is not part of the routine practice in medical oncology, and reports are not generally available in the literature. Therefore, for proper assessment of the impact of dosing schedules in clinical trials, it is critical that such data be provided.

Calculations of the impact of dose intensity on outcome are particularly important in estimating the value and exploring some of the pitfalls of adjuvant chemotherapy. The steep dose-response curve for anticancer drugs indicates that dose reductions in adjuvant drug treatment programs are likely to be associated with significantly less therapeutic effect. Dose reduction, however, has been the norm in the design of adjuvant trials. One example is the standard CMF regimen for breast cancer referred to in Table 17-3. The initial reports of this regimen revealed an impressive complete remission rate of approximately 30%, albeit at the expense of considerable host toxicity.¹¹⁹ When this regimen was advanced for use in the cooperative group setting, initially for advanced disease and later for adjuvant trials by Bonadonna et al.,¹²⁰ its doses were arbitrarily reduced without pretesting the potential impact of such reductions on clinical outcome. In addition, further reduction was empirically made for patients older than 60 years, with the assumption that such a dose reduction would be required for

TABLE 17-3. Sample Calculations: Dose Intensity, Relative Dose Intensity, and Average Relative Dose Intensity

CALCULATION OF RELATIVE DOSE INTENSITY (RDI)

Standard: Cyclophosphamide, 80 mg/m²/d (continuously), 560 mg/m²/wk

Test schedule: Cyclophosphamide, 100 mg/m²/d (days 1-14, q28d), 350 mg/m²/wk

$$RDI = 350/560 = 0.62$$

CALCULATION OF AVERAGE RDI

Standard:

Cyclophosphamide, 2 mg/kg/d, 560 mg/m²/wk

Methotrexate, 0.7 mg/kg/wk, 28 mg/m²/wk

5-Fluorouracil, 12 mg/kg/wk, 480 mg/m²/wk

Test regimen:

Cyclophosphamide, 100 mg/m²/d (days 1-14), 350 mg/m²/wk; RDI = 350/560 = 0.62

Methotrexate, 40 mg/m² on days 1 and 8, 20 mg/m²/wk; RDI = 20/28 = 0.71

5-Fluorouracil, 600 mg/m² on days 1 and 8, 300 mg/m²/wk; RDI = 300/480 = 0.62

Repeat cycles q28d

$$\text{Average RDI} = (0.62 + 0.71 + 0.62)/3 = 0.65$$

*Assume standard regimen to be cyclophosphamide, methotrexate, and 5-fluorouracil. To convert milligrams per kilogram (mg/kg) to milligrams per square meter (mg/m²), multiply by 40.¹¹⁵

age. When the effect of these reductions is correlated with outcome, there is a strong suggestion of a negative impact.^{120,121} In premenopausal women, the differences in relapse-free survival at both low and high doses of CMF are statistically significant. The importance of dose effect was further confirmed by a large study in which a survival benefit was observed as a result of increasing dose intensity in the adjuvant chemotherapy for women with stage II, node-positive breast cancer.¹²²

An increase in the dose intensity represents one approach to improve on the effect of specific drugs or drug combination, but it may not be useful in all clinical circumstances. In the setting of large tumor burdens, the dose-response curve tends to shift to the right. At the low end of the curability curve (i.e., in the presence of the highest tumor burdens), an increase in dose intensity may not improve treatment outcome, as the dose-response curve is flat, but most often leads to unacceptable host toxicity. In addition, increasing the dose intensity of drug regimens that are already associated with curing nearly 100% of a subset of patients would not be expected to be of clinical benefit. Such a scenario would hold for the treatment of germ cell cancer using the cisplatin, etoposide, bleomycin combination and for Hodgkin's disease, using either the mechlorethamine, vincristine (Oncovin), procarbazine, and prednisone regimen; the doxorubicin, bleomycin, vinblastine, and dacarbazine regimen; or regimens derived from them, such as BEACOPP (see Chapter 45.3). However, for most drugs, there appears to be a threshold dose that produces clinical response. The success of high-dose chemotherapy programs with stem cell support in refractory lymphomas, breast cancer, childhood sarcomas, and neuroblastomas suggests that maximizing dose intensity can improve response rates or cure in drug-responsive tumors.

Frei et al.¹²³ and Hryniuk et al.¹²⁴ have proposed the term *summation dose intensity* to reflect the relationship between dose and combination chemotherapy. As part of this concept, they suggested that the final outcome of either a high-dose or combination treatment must be related in some manner to the sum of the dose intensities of all the agents used in that treatment. The intrinsic chemosensitivity of a given tumor is critical for treatment success. An active agent is defined as one that, when used alone, is associated with at least a 30% response rate for a given tumor. It is now well appreciated that for almost all malignancies, a combination regimen incorporating at least three active drugs is necessary for cure. In the case of childhood leukemia, the cure rate increases linearly when the number of active drugs increases from three to seven. The critical issue for this concept is that all active agents must be used at their full therapeutic doses. However, until the advent of the various cytokine growth factors and autologous or peripheral stem cell transplantation (or both), the effective administration of maximal doses of chemotherapy has not been possible. Although the concept of summation dose intensity is not new, it does offer a unified approach for the careful design and interpretation of clinical trials.

IN VITRO DRUG-RESPONSE ASSAYS

Several methods have been developed since the 1950s to determine the *in vitro* drug sensitivities of human tumor cells to various anticancer agents.¹²⁵⁻¹³⁹ The advent of reliable *in vitro*

drug-response assays has raised the possibility of selecting effective anticancer agents to be used either alone or in combination to treat a patient's individual tumor. In this setting, identification of agents with an extremely low probability of response makes it possible to eliminate the use of such agents and thus their potential for adverse events. A number of methods have been used to investigate the sensitivity of tumors and tumor cell lines, including clonogenic, differential staining cytotoxicity assay; colorimetric, ³H-thymidine incorporation assay; and chemotherapeutic treatment of athymic nude mice with human tumor xenografts. Hausf and Bosanquet¹⁴⁰ reviewed the correlation between results of *in vitro* sensitivity testing and a patient's tumor response to chemotherapy and, in general, they found an overall sensitivity of 85% and an overall specificity of 80%.

In the mid-1950s, Black and Spear^{134,135} were the first to report the use of an *in vitro* assay to predict patient response. Their studies compared the *in vitro* activity of aminopterin with its clinical response. Their assay technology was based on colorimetric detection of viable cells using a substrate for mitochondrial succinate dehydrogenase. Although the predictive accuracy of their results was not particularly strong, the development of the clonogenic stem cell assay in the 1970s brought *in vitro* testing of solid tumors into the mainstream.¹³⁷ However, the results of these studies indicated that there were significant technical issues to overcome.^{138,139} As illustrated in Table 17-5, further work to improve on the technology led to a variety of techniques and approaches with a pronounced ability to identify drug resistance accurately. The major distinction among differing assay methods is the end point used to measure viability. Assay end points include colony growth from stem cells, incorporation of tritiated thymidine, microscopic examination of cells with vital dyes, mitochondrial enzyme activity, cytosolic esterase activity, and adenosine triphosphate content. Given the variety of assay types, it is remarkable that the predictive accuracy for the identification of chemosensitive tumors using most of these approaches appears to be at least 90%. Several issues should be considered when evaluating an assay technology (Table 17-5).^{125,127,140}

The clonogenic assay evaluates the ability of chemotherapeutic agents to inhibit tumor stem cell proliferation in culture, using a medium that precludes proliferation of nontransformed cells.^{133,134} Most of the *in vitro* drug-response techniques use similar methods for tumor preparation. Solid tumors are disaggregated into suspensions of multicellular clumps with scissors or by passing the fragments through mesh or by stirring tissue fragments with collagenase. Single-cell suspensions then are generated by passing the cellular aggregates through high-gauge needles.^{133,138} Cell suspensions are incubated with drug for 1 hour, rinsed, and plated on an agar base with growth medium. After a period of 14 to 28 days, the number of colonies that have grown from the treated cells is compared with the number of colonies from untreated control cells. The fraction of colonies that grow provides an index of drug activity. Studies by the National Cancer Institute and the Southwest Oncology Group indicate that the assay is reproducible among multiple laboratories.¹³⁸ Problems with assay interpretation arising from the serial plating of small cell clumps were overcome with the use of chromomycin A3.^{138,139}

The conventional clonogenic stem cell assay has suffered from a relatively low success rate (<50%) of specimens yielding results, rendering it difficult to accrue adequate numbers

TABLE 17-4. Correlations of *In Vitro* Test Results with Patient Response

Assay Type	Patients	TP	TN	FP	FN	Predictive Accuracy ^a		Sensitivity (%) ^b	Specificity (%) ^c
						+	-		
Clonogenic	2300	512	1427	226	135	69	91	79	86
5 d thymidine	494	123	432	119	20	51	92	86	66
3 h thymidine	171	90	40	21	20	81	67	82	66
DiSC	510	247	175	72	16	77	92	94	71
MTT	326	187	74	37	28	83	73	87	67
ATP	129	74	37	6	12	93	76	86	86
FCA	333	154	116	52	11	75	91	93	69
Total	4263	1387	2101	533	242	72	90	85	80

ATP, adenosine triphosphate; DiSC, differential staining cytotoxicity; FCA, fluorescent cytoprint assay; FN, patients who are resistant *in vitro* but respond clinically; FP, patients who are sensitive *in vitro* but resistant clinically; MTT, tetrazolium dye; TN, patients who are resistant *in vitro* and do not respond to chemotherapy; TP, patients who are sensitive *in vitro* and respond to therapy.

^aPredictive accuracy: + indicates TP/(TP - FP), percentage of patients with sensitivity in the test who respond; - indicates TN/(TN + FN), percentage of patients with resistance in the test who do not respond to therapy.

^bTest's ability to detect clinically responsive patients.

^cTest's ability to detect clinically unresponsive patients; clinical response is greater than or equal to a 50% reduction in assessable disease.³⁵

Note: Summary of clinical correlations is pooled from individual studies referenced in text.

patients into clinical trials.¹³³ Although this factor initially dampened enthusiasm for this approach, a significant number of clonogenic assays (>2500 cases) have now been performed by various groups, with an overall positive predictive value of 69% and a negative predictive value of 91% (see Table 17-4).¹²⁰

Tritiated thymidine incorporation, as an assay end point, was introduced in part to eliminate the problem of discriminating between true colony growth from a single cell and from a clump of cells plated at the outset. This technique also decreased the assay time from more than 14 days to less than 1 week and was associated with an improved success rate of diagnostic yield to 85%.^{125-127,141,142}

Processing and plating of the tumor for the thymidine assay is similar to that for the clonogenic assay. However, in the thymidine assay, small clumps rather than single cells are preferred to maintain cell-cell interactions. In addition, cells are grown in an agar suspension, which allows tumor growth *in vitro* to recapitulate the three-dimensional *in vivo* morphology. Cell-cell interactions resulting from three-dimensional growth may be critical for the detection of acquired drug resistance, which can be missed in monolayer cultures.¹⁴³

In contrast to the clonogenic assay, prolonged drug exposures are utilized in the thymidine-based system. Tumor suspensions are continuously exposed to drug for 5 days, and tritiated thymidine is added during the final 48 hours of the assay to label proliferating cells. Determination of drug action is based on a comparison of the incorporation of labeled thymidine by untreated controls with incorporation by the groups treated with different drugs. Clinical correlations obtained using this assay technique demonstrate a reasonable overall predictive accuracy (72%) and indicate that it is an accurate predictor of drug resistance (99%).¹²⁶ The prolonged drug exposure in the thymidine assay results in a five- to 20-fold higher concentration × time factor than that used in the clonogenic assay, biasing assay accuracy toward detection of drug resistance. Tumor growth after drug exposure in the thymidine assay is associated with multifold drug resistance, which led the

authors of one article to describe it as the "extreme drug resistance assay."¹²⁶ Some paclitaxel-resistant tumors identified with this technique have been found to overexpress P170 glycoprotein, suggesting that this assay can be used to identify the activity of specific mechanisms of drug resistance in different tumor histologies.¹⁴⁴

Another promising assay is the differential staining cytotoxicity (DiSC) assay.^{140,145} The DiSC assay relies on the structural integrity of cells. In the DiSC assay, cells are incubated with drugs for 4 days. Dead cells are stained in suspension with fast green dye in the absence or presence of nigrosin, and duck red blood cells are added as an internal standard for counting. The specimen is cytocentrifuged to deliver discs of cells onto microscope slides. Live cells then are stained with either hematoxylin-eosin or Romanowsky stain. The end point of this test is the morphologic identification of tumor cell cytotoxicity as compared with the internal control of fixed duck erythrocytes. The DiSC assay requires more than 10% tumor cells and measures cell kill in both dividing and nondividing tumor cell populations. Microscopic identification of the cell population renders it possible to determine the differential kill of normal and tumor cells,

TABLE 17-5. Factors Influencing the Utility of the *In Vitro* Assay

Tumor heterogeneity: Is the assay end point selective for malignant cells versus stromal cells?
Is the assessability rate greater than 80%?
Have the assays been correlated with clinical response and survival?
Can the tests evaluate all histologic types, or are they restricted to only certain types of tumors?
Are clinically appropriate drugs evaluated in the test?
Does the turnaround time meet clinical requirements?
Is the test information easily interpreted and applied?
Is the test cost-effective?

and this is the therapeutic index for new agents undergoing *in vitro* screening for activity. The DiSC assay (see Table 17-4) offers an overall predictive accuracy of 83%, with a sensitivity of 94% and a specificity of 71%.^{125,132,145}

The potential efficacy of individualized chemotherapy selected by *in vitro* drug sensitivity testing for patients with cancer has been reviewed.^{146,147} A number of issues seriously limit the widespread use of this approach in the clinic. First, *in vitro* drug sensitivity testing is relatively expensive and time-consuming. Second, the efficient procurement of tumor tissue remains a serious problem. In fact, only two studies, both from the National Cancer Institute, have evaluated the ability to obtain tumor tissue from patients with limited- and extensive-stage small cell lung cancer.^{147,148} Tumor tissue was obtained from 30% of patients with limited-stage disease, in contrast to nearly 70% of patients with extensive-stage disease. Third, even with successful procurement of tumor tissue, a host of technical issues limits the ability for efficient and successful drug testing. In fact, of 12 different trials reviewed, only slightly more than one-half of all tumor samples had sufficient cell numbers for drug testing. Finally, only one-third of all patients entered in prospective trials of *in vitro* drug testing were actually treated with an *in vitro* best regimen. In those patients, the response rates appear to be as good as, and perhaps even slightly better than, those achieved with empiric therapy. It is not surprising, then, that when all the clinical studies are taken together, no potential benefit in survival is observed for this approach. Of note, however, is a survival advantage that has been reported, in a small select series of studies, in patients treated with an individualized *in vitro* best regimen.¹⁴⁸

The reliability of newer *in vitro* assay technologies to identify drug sensitivity suggests that such assays can help the clinician to avoid exposure of patients to the toxicity of drugs with little clinical benefit. Although the promise of an *in vitro* sensitivity assay has not yet been met, there remains value in identifying inactive agents before their administration and eliminating them from drug combinations. These tests render it possible to tailor drug combinations for the individual cancer patient. They also offer a rational stopping point for both the patient and clinician in situations in which the patient's tumor demonstrates extreme resistance to all conventional anticancer agents. An understanding of when to terminate therapy in hopeless situations is as important as any management issue facing the clinician.¹⁴⁹ Although only a few hundred patients have been enrolled to date to evaluate the impact of *in vitro* assay-directed therapy on survival, it is intuitively obvious that there should be a therapeutic advantage in the activity of agents to which a tumor is highly responsive *in vitro* as compared with agents that demonstrate significant *in vitro* drug resistance.¹²⁵ Further prospective, randomized studies are needed to define more properly the true role of *in vitro* drug testing in the selection of chemotherapy for cancer patients in the adjuvant, induction, or salvage setting.

Although *in vitro* tissue culture studies serve as an important guide for selecting chemotherapy, they are inadequate at addressing the issues of tumor cell heterogeneity, drug distribution, drug bioactivation, and host toxicity. *In vivo* model systems overcome some of these obstacles, and several have been developed, including the subrenal capsule assay, a semipermeable membrane in the Millipore diffusion chamber as vessels for tumor implantation into mice, and the tumor xenograft model, which is perhaps the most widely used method for drug testing.^{140,150-152} However, each of these experimental systems has

its unique drawbacks. Recently, a novel system was developed using a semipermeable polysulfone fiber with a molecular weight cutoff of 30 kD. Human cancer cells derived from tissue culture or from patient tumor specimens are injected directly into semipermeable fibers that are then implanted into immunocompetent rats.^{153,154} Animals are treated with the given drug, and, after a defined period, they are sacrificed, the fibers are recovered, and the remaining viable cells are counted using trypan dye exclusion method. There are several advantages to this polysulfone fiber model. First, the entire process of tumor recovery, injection, and implantation of fibers, drug treatment, fiber recovery, and cell analysis can be completed in less than one week. This short period minimizes the potential waiting time for selection of the optimal drug, thereby rendering this model feasible for application in the clinical setting in treating the individual patient. Second, the results from this model system are consistent, reliable, and highly reproducible. As many as six to eight fibers can be implanted into an individual rat; thus, each rat can be injected with the same cell type and the individual rat treated with the same drug. In addition, this reduces the unnecessary expense of using multiple animals for drug *in vivo* testing. Third, because up to six to seven fibers can be implanted into an individual rat, cancer cells derived from different primary tumors can be tested simultaneously for drug sensitivity. This process that can result in greater cost and time efficiency. Further testing and validation are required to determine whether such a novel *in vivo* system can help to individualize and optimize the clinical therapy of cancer patients.

Finally, studies by Waldman et al.¹⁵⁵ have raised concerns regarding the validity of the *in vitro* colony formation assay as a measure of the cytotoxicity of DNA-damaging agents in tumor cells with altered checkpoint response. Using the human colon cancer HCT116 cell line that expresses wild-type p53 and (p21+/+) and a subline that was rendered p21-deficient (p21-/-) by homologous recombination, these researchers tested the effects of γ -irradiation using the *in vitro* colony formation assay and an *in vivo* xenograft model. Of note, they observed no differences in sensitivity to ionizing radiation, as determined by the *in vitro* colony formation assay. However, in the nude mouse xenograft model, they found that tumors derived from the parent p21+/+ cell line were able to survive after exposure to ionizing radiation. In contrast, a significant reduction in the size of the tumors deficient in p21 underwent apoptosis and were thus completely cured. Clearly, the *in vitro* assay was unable to detect this significant difference in sensitivity, as the processes of cell arrest and apoptosis preclude the growth of colonies. Given the critical role of checkpoint status as a determinant of chemosensitivity, these findings are important as they suggest that *in vivo* assays may represent a more relevant model system to compare the effects of anticancer agents.¹⁵⁶ Moreover, such an *in vivo* model may be ideal for testing novel agents that specifically target cell-cycle control and the pathways associated with the process of apoptosis.

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CHAPTER 18

Principles of Cancer Management: Biologic Therapy

Biologic therapy is cancer treatment that produces antitumor effects primarily through the action of natural host defense mechanisms or the administration of natural mammalian substances. Biologic therapy has emerged as an important fourth modality for the treatment of cancer.* Its increased application is the result of a better understanding of the basic aspects of host defense mechanisms against cancer and rapid biotechnologic developments that made molecules available in quantities large enough for use in manipulating biologic processes *in vivo*. Although this field is still in its infancy, there are many examples of the successful application of biologic therapy to the treatment of human cancers.

BASIC PRINCIPLES OF TUMOR IMMUNOLOGY

Most applications of biologic therapy for cancer have attempted to stimulate immune defense mechanisms. The immune system evolved as a means to detect and eliminate molecules or pathogens that are recognized as "nonself" but not to react to host (self) tissues. Many immunotherapies attempted to cause the tumor to appear more "foreign" compared with normal tissues or tried to magnify relatively weak host immune reactions to growing tumors.

The immune system differs from most other organ systems because its cells are not in constant contact with each other. They

circulate freely throughout the body in and out of the circulatory and lymphatic systems. Immune reactivity involves the integrated action of lymphocytes, monocytes, macrophages, basophils, eosinophils, dendritic cells, endothelial cells, and many other cells throughout the body. Although separate functions have been assigned to these cell types, it is now clear that they interact in many ways and can regulate one another's activities.

Immune cells secrete two major classes of soluble protein. The first of these lymphocyte products to be recognized was the antibody. Antibodies are a group of proteins composed of one or several units, each of which is composed of two pairs of different polypeptide chains (i.e., heavy and light chains). Each unit possesses two recognition sites, which are capable of combining with the immunizing antigen. The unique bond between antigen and antibody is part of the basis for the exquisite specificity that is the hallmark of immunologic reactivity. The existence of circulating antibodies was first demonstrated in 1890, and until recently, scientific studies of antibodies monopolized the study of immune reactions.

Since the 1970s, it has become clear that selected subpopulations of lymphoid cells can secrete a second (nonantibody) class of protein molecules. These molecules are not biochemically similar to antibodies, are produced in tiny amounts, and are not normally detectable in the circulation. Collectively called *cytokines*, they represent a new class of hormones with actions on many different target cells within and outside the immune system. Increasing knowledge of a wide variety of cytokines has dramatically altered the understanding of the functions of the immune system and created new possibilities for cancer immunotherapy.

*For a definitive compendium of information on the biologic therapy of cancer, refer to the companion volume, Rosenberg SA, ed. *Principles and practice of the biologic therapy of cancer*. Philadelphia: Lippincott Williams & Wilkins, 2000.

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